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FINAL

DRINKING WATER CRITERIA DOCUMENT

FOR

DINOSEB

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Health and Ecological Criteria Division
Office of Science and Technology
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U.S. Environmental Protection Agency
Washington, DC 20460

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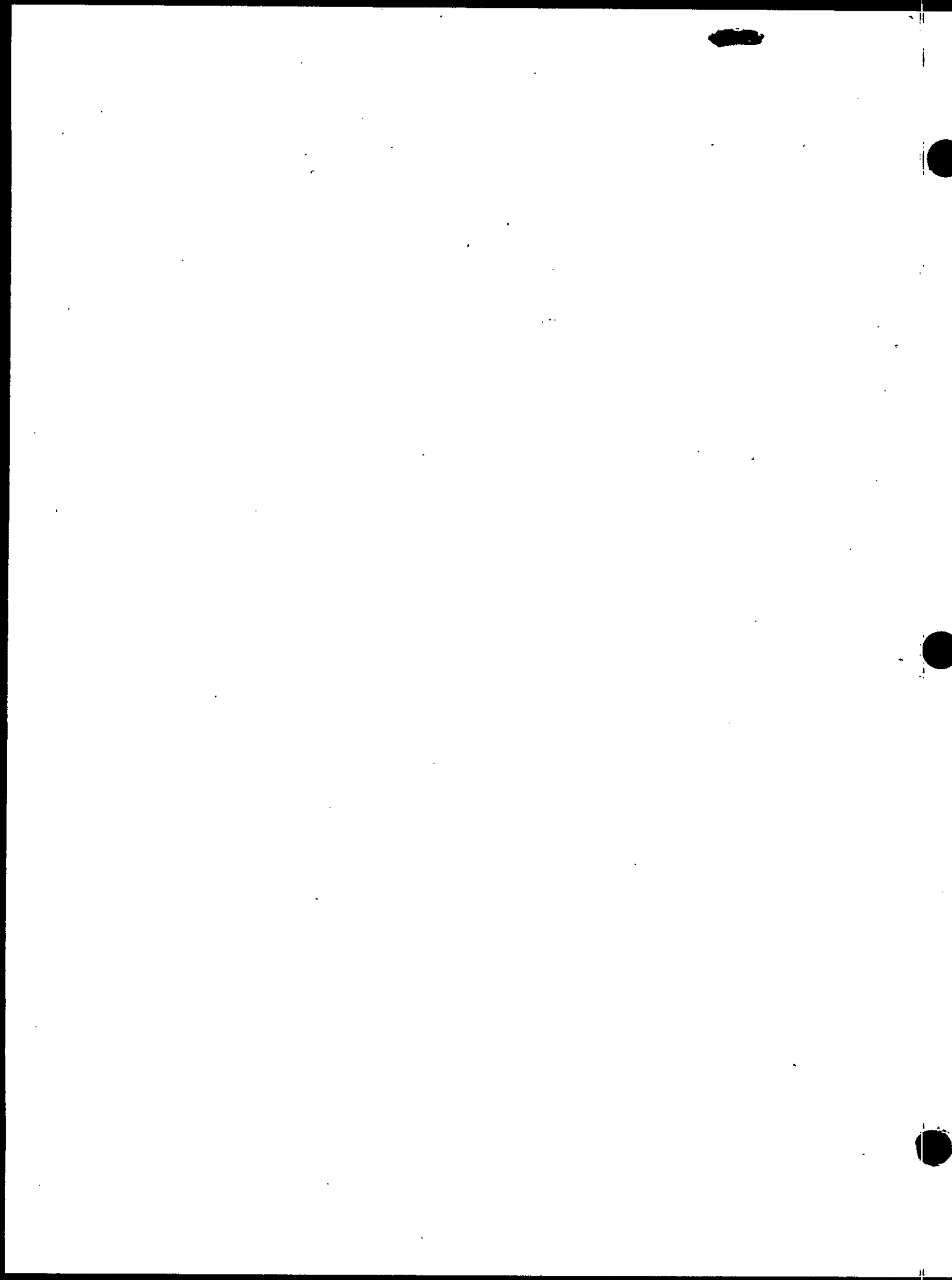


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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish Maximum Contaminant Level Goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology, and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to April 1987; however, more recent data have been added during the review process and in response to public comments.

When adequate health effects data exist, Health Advisory values for less-than-lifetime exposures (One-day, Ten-day, and Longer-term, approximately 10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

James R. Elder
Director
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I. SUMMARY

Dinoseb, 2-sec-butyl-4,6-dinitrophenol (DNBP), is poorly soluble in water (0.52 g/L) but is readily soluble in alcohol and other organic solvents. DNBP is often used in salt form as both a pre- and postemergence herbicide for a wide variety of crops.

About 25 to 37% of an oral dose and approximately 40% of an intraperitoneal dose of dinoseb is excreted in the feces (the remainder being eliminated in the urine or retained in tissues), suggesting that fecal excretion results primarily from secretion of absorbed dinoseb into the intestines. On the basis of this information, absorption may be estimated to be essentially complete. A maximum of approximately 7% of the administered dose was found in the urine of monkeys following percutaneous administration of dinoseb. When administered orally to pregnant mice, absorbed dinoseb and/or dinoseb residues are distributed to all tissues. Residue levels in the brains and embryos of pregnant mice never exceeded 2.5% of the plasma level. Dinoseb is extensively metabolized by several pathways in animals: (1) one or both of the nitro groups can be reduced to the amine, which may then be acetylated; (2) the terminal methyl groups of the side chain can be oxidized to carboxyl groups; and (3) the compound or its metabolites may be conjugated, primarily as glucuronides. Although a number of metabolites have been identified, a greater number have been detected but not yet characterized. Dinoseb and its metabolites are excreted in both urine and feces, with low amounts in the bile. Excretion was monophasic following a single oral dose, with a half-time of about 35 hours. A $t_{1/2}$ of about 8 hours was observed following an intraperitoneal dose.

Acute oral LD_{50} values for the rat, rabbit, mouse, and guinea pig range from 14 to 114 mg/kg. The intraperitoneal LD_{50} value has been reported as 20.2 mg/kg for female mice and 10 mg/kg for male mice. Dinoseb is well absorbed through intact skin, with dermal LD_{50} values in the rat ranging from 67 to 134 mg/kg.

When administered intraperitoneally, dinoseb doses of 12 to 16 mg/kg/day for 5 days intensified inhibitory and excitatory activities in the brains of rats, while doses of 2 to 8 mg/kg/day for 5 days were without effect. In ducklings, dinoseb and a number of other dinitrophenols have the ability to produce cataracts following dietary exposure.

Increased mortality was noted in rats fed diets containing dinoseb at 300 ppm and above for 60 days. Decreased body weight gains were noted at dietary levels of 50 to 200 ppm. Diffuse tubular atrophy of the testes was noted in males receiving 200 ppm. In a 6-month feeding study, the body weights of rats receiving 5.4 mg/kg/day were slightly lower than those of controls at the end of the treatment period. No other toxic effects were noted in these animals, except for a slight but statistically significant increase in liver weight. Dietary levels of 13.5 mg/kg/day caused increased mortality, whereas 2.5 mg/kg/day was a No-Observed-Adverse-Effect Level (NOAEL).

No adverse effects were noted in beagles administered diets containing 0.01 or 0.005% dinoseb for 91 days. However, in females fed dietary levels of 0.02 and 0.03%, growth retardation, increased average liver weights, mural endocarditis, and microscopic heart changes were noted. The NOAEL was established at 0.01% (100 ppm), equivalent to 4 mg/kg/day.

A compound-related decrease in mean thyroid weights was noted in rats receiving dietary levels of 1, 3, and 10 mg/kg/day for 2 years. No other compound-related effects were noted, but histopathologic examination of tissues was conducted in only a limited number of animals. A Lowest-Observed-Adverse-Effect Level (LOAEL) of 1 mg/kg/day was established from this study.

Mice orally administered dinoseb for 100 weeks at dietary levels of 1, 3, and 10 mg/kg/day showed cystic endometrial hyperplasia and atrophy, hypospermato-genesis, and testicular degeneration, but oncogenic effects were equivocal. Statistically significant increases in liver adenomas and adenomas plus carcinomas were observed in female mice only. Lenticular opacities were

observed at the 3- and 10-mg/kg dose levels, but animals receiving the low dose were not examined. A systemic NOAEL is less than 1 mg/kg/day.

Dinoseb has been found to be teratogenic in several species including rabbits, rats, and mice following oral, dermal, intraperitoneal, and subcutaneous administration to pregnant animals. Oral administration of dinoseb in mice on day 8 of gestation, at doses of 26 and 33 mg/kg, produced supernumerary ribs. The same anomaly was seen in rats administered 10 mg/kg dinoseb on days 6 to 15 of gestation. Skeletal anomalies, as well as external and visceral malformations, were also observed in rabbits orally administered dinoseb on days 6 to 18 of gestation at the same dose of 10 mg/kg.

Oral administration of dinoseb on days 10 to 12 of gestation produced skeletal anomalies at 32 mg/kg/day. Some anomalies occurred at 20 mg/kg, but these were considered marginal. Treatment of pregnant mice with 17.7 mg/kg dinoseb administered intraperitoneally on days 10 to 12 of gestation resulted in fused or missing ribs, fused or missing sternbrae, fused or unossified or absent vertebrae, and absent or unossified long bones. Subcutaneous doses produced comparable effects, but these were observed at somewhat higher dose levels.

The developmental anomalies appeared at lower dosages of dinoseb after dermal exposure than after oral administration. In a developmental toxicity study in rabbits, a developmental NOAEL of 1 mg/kg was identified based on increases in gross external, soft tissue, and skeletal malformations in the fetuses of dams given 3 or 9 mg/kg/day percutaneously on days 7 through 19 of gestation. These malformations included hydrocephaly, microphthalmia, anophthalmia, craniosynostosis, and small eye sockets. The maternal NOAEL was also 1 mg/kg/day based on increased mortality, slight decreases in body weights during the dosing period, and increased incidences of gross lesions upon necropsy (hemorrhaging in brain, trachea, thymus, lungs, and subdermis of the thorax and abdomen) of rabbits receiving dosages of 3 mg/kg/day or higher.

The teratogenic effects noted in mice following intraperitoneal administration of dinoseb are diminished by its metabolism. Compounds that stimulate drug metabolism (such as phenobarbital) have been shown to decrease dinoseb toxicity. Conversely, inhibitors of drug metabolism (such as SKF-525A) potentiate dinoseb-induced teratogenicity.

Other studies suggest that the rat may be more susceptible than the mouse to the effects of dinoseb. Pregnant Sprague-Dawley rats fed 9.23 mg/kg/day of dinoseb in the diet on days 6 to 15 of gestation had poor weight gain, ataxia, and lethargy. At all dose levels above 8.60 mg/kg/day, there was a significant reduction in fetal survival per litter at birth.

Results of a study of the postnatal morphology and functional capacity of kidneys in neonates of Sprague-Dawley rats treated intraperitoneally with dinoseb show that approximately 40% of the fetuses of mothers treated with dinoseb at 8 to 9 mg/kg/day had dilated renal pelvises and/or ureters. Histological examination revealed relatively complete recovery at 6 weeks of age. In contrast, livers of fetuses from this same group showed highly vacuolated cells that were still present in offspring 6 weeks later, along with necrotic cells and pyknotic or karyorrhectic nuclei in other cells, thus demonstrating little evidence of recovery.

In mice orally administered 15 and 100 mg/kg during days 8 to 12 of gestation, no effects were seen on postnatal parameters at day 22 or 30.

In a three-generation rat study in which dinoseb was administered at 1, 3, and 10 mg/kg/day, no effect on survival, fertility, or fecundity was seen. At 10 mg/kg/day, the number of pups born and the pup weights at weaning were lower and were attributed to maternal toxicity.

In an 11-week study with rats, dinoseb at dietary levels of 15.6 or 22.2 mg/kg/day produced marked oligospermia and extensive loss of spermatogenic cells. Little recovery occurred during 16 weeks following cessation of exposure. At 9.1 mg/kg/day, decreased epididymal sperm counts, atypical

epididymal spermatozoa, and minimal testicular changes were present that appeared to be reversible. No effects were seen in rats fed 3.8 mg/kg/day for 11 weeks. In a study on testicular toxicity of dinoseb in mice, daily intraperitoneal injections of 20 mg/kg/day for 5 consecutive days produced no testicular effects.

A number of assays were conducted to determine the mutagenic potential of dinoseb. Negative responses were elicited in the Ames assay with Salmonella typhimurium and Escherichia coli, sex-linked recessive lethal assay in Drosophila melanogaster, mitotic recombination assay in Saccharomyces cerevisiae, and unscheduled DNA synthesis assay in human fibroblasts. However, positive responses were elicited in DNA repair synthesis assays using repair-deficient and repair-proficient strains of E. coli, Bacillus subtilis, and S. typhimurium. Dinoseb also induced small increases in mutation frequencies in a mouse lymphoma cell line.

No increases in the incidence of tumors were found in rats fed diets containing 1, 3, or 10 mg/kg/day of dinoseb for 104 weeks. However, limited histopathology was performed for this study; thus, the oncogenic potential was difficult to assess. Mice orally administered dinoseb for 100 weeks at dietary levels of 1, 3, and 10 mg/kg/day showed equivocal oncogenic effects, although statistically significant increases in the incidence of liver adenomas and combined adenomas and carcinomas were observed in female mice only. A systemic NOEL of less than 1 mg/kg/day was calculated based on increased incidences of cystic endometrial hyperplasia and atrophy/hypospermatogenesis/degeneration in the testes of dosed animals.

Only one case study of dinoseb poisoning in humans was identified in the available literature. Signs of toxicity appeared shortly after the individual had applied the compound to a field. The individual wore a gauze face mask but not gloves as he repaired plugged spray-jets in the field. Thus, both skin and inhalation exposure may have been extensive. Elevated body temperature, liver damage, and subsequent lung involvement were the major effects. The liver damage appeared to be particularly long lasting.

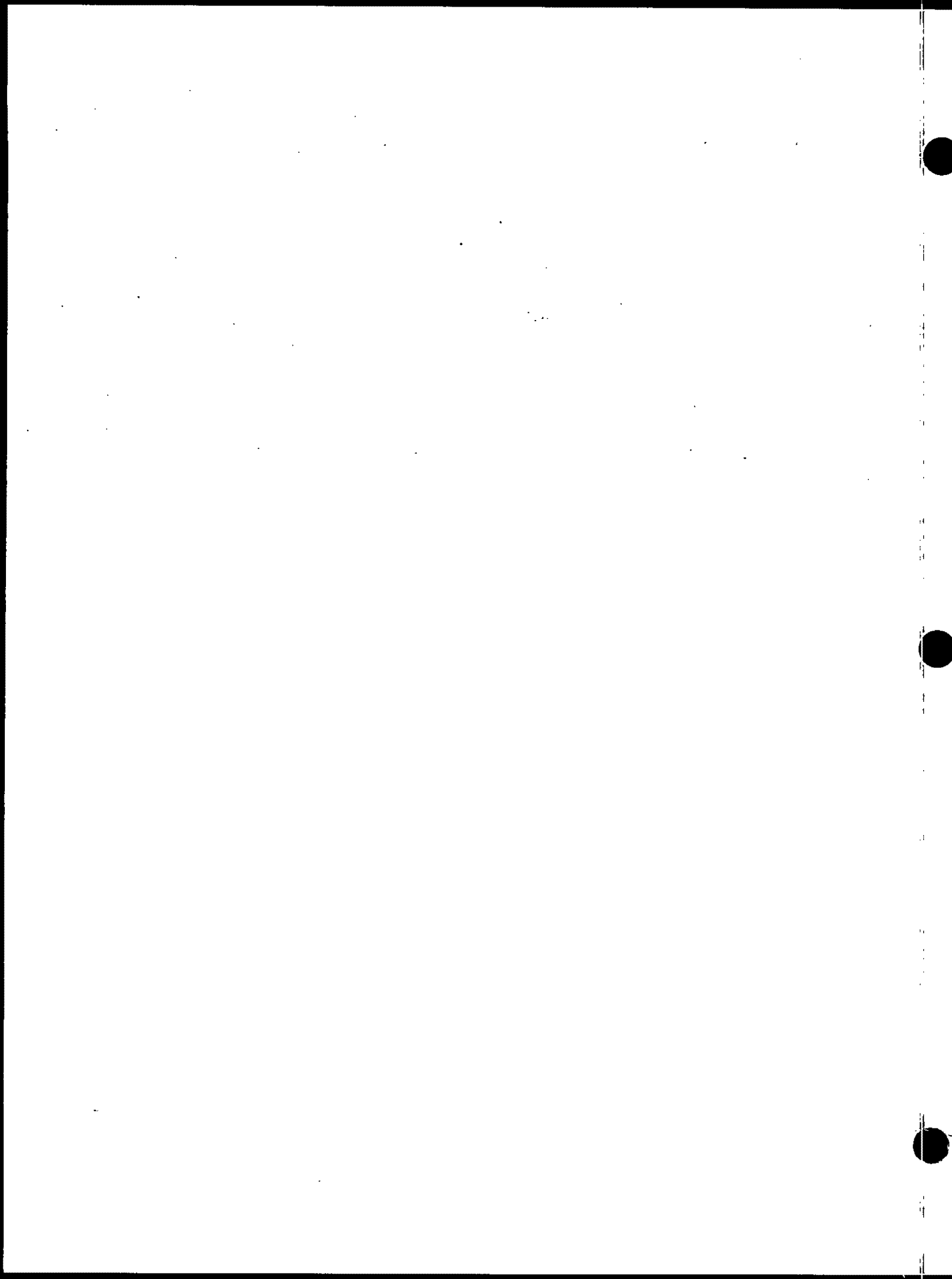
No relevant epidemiological studies were located. Estimates of dermal and inhalation exposure in applicators were reported to be 33.7 and 0.12 mg/70-kg man/hour. Dinoseb has been detected in the blood of workers employed in the manufacture of the herbicide at levels ranging from 0.0 to 0.2 μ g/mL blood.

The toxicity of dinoseb was suggested to be due to the uncoupling of oxidative phosphorylation. Experimental studies demonstrated that dinoseb inhibition of brain oxidative phosphorylation correlated with signs and symptoms of toxicity. In mice showing severe signs of poisoning, oxidative phosphorylation of brain mitochondria was completely inhibited.

There were no suitable studies available for calculation of the One-day Health Advisory. Thus, the Ten-day HA value was used as a conservative estimate for the One-day HA. A developmental NOAEL of 3.0 mg/kg/day, based on absence of teratogenic effects in fetuses of pregnant rabbits exposed by oral administration on days 6 to 15 of gestation, was used to calculate the Ten-day HA value of 300 μ g/L for a 10-kg child. A Lowest-Observed-Adverse-Effect Level (LOAEL) of 1.0 mg/kg/day, based on a decrease in pup body weight at all levels in a two-generation study, was employed to calculate the Longer-term HA of 10 μ g/L for a 10-kg child and 40 μ g/L for a 70-kg adult. Both a 100-week feeding study with mice and a 2-year feeding study with rats indicate NOAEL levels below 1 mg/kg/day. Treatment-related cystic endometrial hyperplasia and atrophy, hypospermatogenesis, and degeneration of the testes were noted in dosed mice, whereas decreased thyroid weights were noted in rats. Therefore, a LOAEL of 1 mg/kg/day was selected to calculate the Reference Dose (RfD) and Drinking Water Equivalent Level (DWEL). The RfD is calculated to be 1 μ g/kg/day, and the DWEL is 40 μ g/L based on these studies.

No calculation of excess cancer risk has been made, since only equivocal long-term effects of dinoseb carcinogenicity have been reported. The only standards or guidelines found were the EPA RfD Work Group approval of a 0.001-mg/kg/day RfD for dinoseb (U.S. EPA, 1987a) and a published tolerance (U.S. EPA, 1986b) for dinoseb of 0.1 ppm for a wide variety of agricultural

commodities. EPA has issued a notice of intent to cancel registration of pesticide products containing dinoseb and dinoseb salts (U.S. EPA, 1986c), as well as an emergency suspension of pesticide products containing dinoseb salts (U.S. EPA, 1986d).



II. PHYSICAL AND CHEMICAL PROPERTIES

A. GENERAL PROPERTIES

Dinoseb is 2-sec-butyl-4,6-dinitrophenol (DNBP). The phenol form is poorly soluble in water (0.52 g/L), but is readily soluble in most organic solvents. The major physical and chemical properties are summarized in Table II-1.

B. MANUFACTURE AND USE

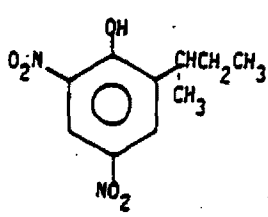
Dinoseb can be synthesized by nitration of 0-sec-butyl phenol, or by sulfonation of phenol to block the p-position, followed by butylation and removal of the sulfonic group (Spencer, 1982). U.S. production of dinoseb was reported to be 6.2 million pounds/year in 1982 (CEH, 1985).

A variety of dinitrophenols are used extensively as herbicides and pesticides. Dinoseb and its salts are used as selective weed killers in field crops and pastures and along roadsides and rights-of-way. Dinoseb ammonium salt is used as a postemergence, selective spray in flax, beans, peas, leeks, potatoes, coffee, vineyards, and orchards. The alkanolamine salt is often used as a preemergence and early postemergence spray (Call et al., 1983). It has been reported that the triethanolamine salt, which is a common formulation, may contain as much as 260 ppm of N-nitrosodiethanolamine (Zweig et al., 1980).

C. ENVIRONMENTAL EFFECT AND STABILITY

Malkomes and Wohler (1983) reported on the effects of dinoseb on microorganisms in two types of soil. Dehydrogenase activity, adenosine triphosphate (ATP) content, and carbon dioxide evolution were used to measure the effect of the herbicide on soil organisms. In laboratory studies, three vessels were filled with about 1 kg of soil, and dinoseb (429 g dinoseb acetate/L) was mixed into the soil at a level equivalent to application of 4 L/hectare. The soils were incubated in the laboratory at 10 or 20°C for

Table II-1. Properties of Dinoseb (2-sec-butyl-4,6-dinitrophenol)

Property	Value
Molecular formula	$C_{10}H_{12}N_2O_5$
Structure	
Molecular weight	240.2
Physical appearance	Dark amber crystals
Melting point	32°C
Density	1.2647 at 45°C
Vapor pressure	(151°C) 1 mmHg (262°C) 100 mmHg
Solubility (g/100 mL solvent)	
Water	0.052
Ethanol	48
Ethyl Ether	Miscible
n-Heptane	27
Toluene	Miscible
Xylene	Miscible

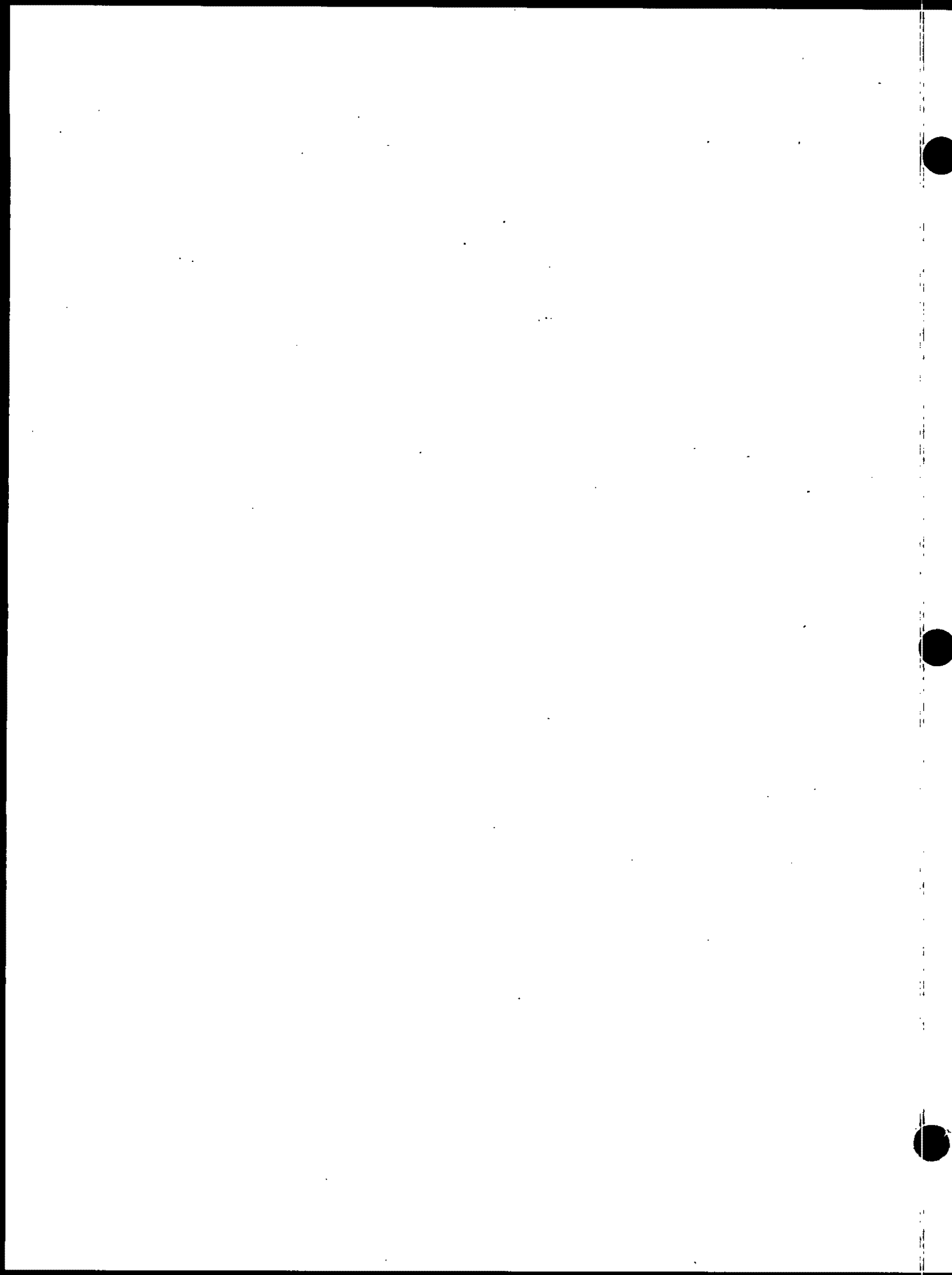
SOURCE: Adapted from the Weed Science Society of America (1983).

periods of up to 16 months. Moisture content was maintained at 40 or 60%, respectively. A marked depression of dehydrogenase activity, ATP content, and carbon dioxide production occurred within 1 to 4 weeks, and this inhibition was still evident after several months. Field studies showed fairly comparable results with respect to dehydrogenase activity (which was still depressed after 2 to 4 months), but other parameters behaved somewhat differently. Carbon dioxide evolution in treated soils varied only slightly from that in control soils, and ATP levels, which sometimes were higher in treated than in control soils, were unpredictable. The authors pointed out the difficulty of predicting the interactions of soil organisms and chemicals.

Hawkins and Sagers (1974) coated eight apples on a tree with ¹⁴C-labeled dinoseb to study retention times under environmental conditions; each apple was exposed to 30 µg of the herbicide. The fruit was harvested at various times (0.5 hours to 28 days) after treatment, and the skin and flesh of the apples were analyzed for radioactivity. Approximately 72% of the dinoseb was lost from the apples by 28 days. The maximum amount of dinoseb absorbed through the skin was 7%. Eight hours after application, essentially all of the residual dinoseb was present on the apple skin in the unaltered state. However, measurements at 8 days and 28 days indicated that most of the label was present on the apple skin in the form of degradation products, indicating transformation of this compound under environmental conditions.

D. SUMMARY

Dinoseb, 2-sec-butyl-4,6-dinitrophenol, is poorly soluble in water (0.52 g/L) but readily soluble in organic solvents. Dinoseb and its salts are used as pre- and postemergence herbicides for a wide variety of crop and noncrop applications. Dinoseb has a relatively long retention time in the environment, although evidence shows that it is degraded when exposed to sunlight and other environmental conditions.



III. TOXICOKINETICS

A. ABSORPTION

Bandal and Casida (1972) administered a single oral dose of 8 to 10 $\mu\text{mol/kg}$ (1.9 to 2.4 mg/kg) of ^{14}C ring-labeled DNBP (99% pure) to male albino rats (180 g) and mice (20 g). After 72 hours, cumulative fecal excretion was 25% of the dose in the rat and about 37% of the dose in the mouse. Elimination in the urine accounted for 64 and 37%, respectively, in the rat and mouse. From these data, absorption may be estimated to be a minimum of 75 and 63% in the rat and mouse, respectively.

Gibson and Rao (1973) administered an oral dose of 32 mg/kg of uniformly ring-labeled ^{14}C -dinoseb to female Swiss-Webster mice. The rate constant for gastrointestinal absorption was estimated to be $7 \pm 4 \text{ hour}^{-1}$ (corresponding to a $t_{1/2}$ of 5.9 minutes). In nine mice, cumulative fecal excretion after 64 hours was 30.4% of the dose, while excretion in bile was 1.4%, suggesting that absorption of dinoseb was approximately 70%. However, fecal excretion was 41% following an intraperitoneal dose (17.7 mg/kg), suggesting that fecal excretion results primarily from biliary excretion of absorbed dinoseb into the intestines. Since the amount in the feces following parenteral dosing (41%) more than accounts for the amount in feces following oral dosing (30.4%), gastrointestinal absorption of dinoseb appears to be essentially complete.

Froslie and Karlog (1970) gave two cows 15 g dinoseb via intraruminal intubation. Within 5 minutes after administration, DNBP could be detected in the plasma at levels as high as 5 to 10 $\mu\text{g/mL}$. Ten days later, 1 to 2 $\mu\text{g/mL}$ of the parent compound persisted in the plasma.

The dermal absorption of ^{14}C -dinoseb was determined following application of a single percutaneous dose of 0.045, 0.2, or 3.6 mg/cm² to a shaved area on the abdomen of female rhesus monkeys (4 to 10 kg body weight, four per group) (Bucks, 1987). An additional four monkeys received single intravenous

injections of 3.0 mg ^{14}C -dinoseb. Each dose contained approximately 5 μCi of radioactivity. After a 24-hour exposure period, the test site was washed to remove the remaining test material, and the monkeys were observed for an additional 13 days during which urine, feces, and blood were collected at specific intervals. The dermal absorption values, determined by measurement of the amount of total radiolabel excreted in the urine for 14 days, were approximately 5.4, 7.2, and 4.9% of the total radioactivity administered at the low-, mid-, and high-dose levels, respectively (Table III-1). This suggests that maximum percent absorption occurred at 0.2 mg/cm² and suggests that there was no further increase in absorption of dinoseb above 0.2 mg/cm².

Bough et al. (1965) reported that dinoseb (technical grade) is readily absorbed through the skin. In four rabbits treated with a dermal application of 50 mg/kg, blood levels rose from 0 mg/100 mL to 6 to 8 mg/100 mL within 2 to 6 hours (resulting in death), but no quantitative estimate of dermal absorption was provided.

B. TISSUE DISTRIBUTION

Gibson and Rao (1973) administered uniformly labeled ^{14}C -dinoseb to pregnant Swiss-Webster mice. Animals received doses of 17.7 mg/kg intraperitoneally or 32 mg/kg by stomach tube on day 11 of gestation. Animals were sacrificed at various time intervals, and tissues (including embryos, placenta, and uterus) were taken for analysis. Radioactivity was present in all tissues examined, but data were presented for only a few tissues and blood plasma. The total ^{14}C residues and unchanged dinoseb in the embryo and blood, liver, and kidneys of pregnant mice 3 hours after oral or intraperitoneal dosing are shown in Table III-2. Both metabolites and parent dinoseb were found in embryos, but embryonic levels examined at various intervals (1 minute to 48 hours) after dosing never exceeded 2.5% of the maternal plasma levels. Brain radioactivity was of the same order of magnitude as that in the embryo, indicating a blood-brain barrier for dinoseb. The volume distribution of dinoseb also appeared to depend on the route of administration. After oral

Table III-1. Percutaneous Absorption of Dinoseb in the Rhesus Monkey

Dose		Absorption	
Appl. Dose (mg/cm ²)	Total Dose (mg)	Max. abs. rate (% dose/hr)	Percent absorption
0.2	2	2.1	5.4
0.045	0.45	0.15	7.2
3.6	32	0.14	4.9

SOURCE: Adapted from Bucks (1987).

Table III-2. Mean Tissue ^{14}C Levels^a of Dinoseb and Metabolites
3 Hours After Oral or Intraperitoneal Administration
to Pregnant Mice

Tissue	Total ^{14}C		Parent dinoseb ^b	
	Oral (32 mg/kg)	ip (17.7 mg/kg)	Oral (32 mg/kg)	ip (17.7 mg/kg)
Blood	31.3 \pm 5.4	45.0 \pm 1.4 ^c	29.7 \pm 4.6	46.6 \pm 3.9
Liver	26.5 \pm 4.1	32.9 \pm 1.3	14.2 \pm 0.6	5.6 \pm 1.8
Kidney	21.8 \pm 3.9	28.6 \pm 4.4	13.8 \pm 1.4	15.4 \pm 1.4
Embryo	2.1 \pm 0.2	5.1 \pm 0.4 ^c	1.8 \pm 0.1	2.9 \pm 0 ^c

^aValues are means \pm SEM for at least three animals. Results are expressed as μg dinoseb/g tissue.

^bMeasured as methyl ethyl ketone-extractable material.

^cp = 0.05 (oral versus intraperitoneal).

SOURCE: Adapted from Gibson and Rao (1973).

administration, dinoseb was distributed in total body water, but after intraperitoneal administration, it was distributed only in extracellular water.

Hall et al. (1978) administered technical grade dinoseb (80%) at 0, 50, 100, 150, 200, 300, 400, or 500 ppm in the diet to Sherman rats (35 to 38 days old) for 60 days. Tissue analysis revealed dose-related tissue residue levels with blood>feces>adipose>brain>liver. Other information was not presented.

C. METABOLISM

Gibson and Rao (1973) also investigated the metabolism of dinoseb in pregnant Swiss-Webster mice. Pregnant mice were dosed orally (32 mg/kg) or intraperitoneally (17.7 mg/kg) on day 11 of gestation with uniformly ring-labeled dinoseb. After 3 hours, animals were sacrificed, and blood, liver, kidney, and embryos were analyzed for the presence of total label and parent dinoseb (methylethyl ketone extractable). The difference between the two values was attributed to metabolites. The data (shown in Table III-2) indicate that dinoseb was metabolized by pregnant mice, and a greater percentage of metabolites was present in tissues (including the embryo) after intraperitoneal dosing than after oral dosing. Specific metabolites were not identified.

Ernst and Bar (1964) studied the metabolites of dinoseb in the urine of rats and rabbits. Rabbits received 15.8 or 20.0 mg/kg DNBP as a single oral dose, and rats received 5.8, 8.9, or 11.5 mg/kg daily. Compounds were identified by paper chromatography in three different solvent systems and by infrared spectroscopy. A small amount of unchanged DNBP (1.9 to 2.7%) was found in the urine of both rats and rabbits. In both the rabbits and rats, 2-(3-butyric acid)-4,6-dinitrophenol, in which the terminal methyl group of the side chain was oxidized to a carboxyl group, was detected; concentrations were higher in the rabbits (10 to 14% of the dose) than in the rats (about 6%). Only the rabbits excreted 2-sec-butyl-4-nitro-6-aminophenyl-O-glucuronide. Total identified metabolites accounted for approximately 15 to 27%

of the dose administered to the rabbits and approximately 6% of the dose administered to the rats. An unidentified compound (substance IV) constituted 15 to 18.5% of the radioactivity in the urine of the rabbits and up to 8% in that of the rats. Ernst (1968) reviewed the metabolic data available on dinoseb and proposed a metabolic scheme for the compound.

Bandal and Casida (1972) studied the metabolism of DNBP in rats and mice. This report also included data on the metabolism of 2-sec-butyl-4,6-dinitrophenyl isopropyl carbonate (dinobuton). Male albino mice (20 g) and rats (180 g), strains not specified, were dosed by stomach tube with 8 to 10 μ mol/kg (1.9 to 2.4 mg/kg) of 14 C-labeled DNBP dissolved in dimethyl sulfoxide or methanol. Treated animals were held in individual metabolism cages for 72 hours, with food and water ad libitum. In both rats and mice, the major portion of a dose of dinobuton was rapidly hydrolyzed to dinoseb, after which both compounds were metabolized by the same pathway. Oxidation of either of the two methyl groups in the sec-butyl moiety may occur, yielding 2-(2-butyric acid)-4,6-dinitrophenol or 2-(3-butyric acid)-4,6-dinitrophenol. The latter was found in the conjugated form in the rat, but not in the mouse. The ortho nitro group may be reduced to yield 2-sec-butyl-4-nitro-6-amino-phenol, which may exist in both free and conjugated forms. Approximately 12 other metabolites were detected but not identified. An additional unknown complex, consisting of at least five metabolites that remained uncleaved by beta-glucuronidase hydrolysis in the rat, accounted for 70 and 52% of the radiolabel recovered in the urine of the mouse and rat, respectively.

Froslie and Karlog (1970) studied the metabolism of DNBP in the cow. A dose of 15 g was administered by tube to the rumen. This was nearly fatal, but the animal slowly recovered over a 2-week period. Analysis of products in the rumen indicated that within 30 minutes, DNBP was converted primarily to 6-amino-NBP. The 6-amino-NBP, in turn, was gradually converted to diamino-BP so that within 4 hours diamino-BP was the only product present in the rumen.

Based on the metabolic data reviewed above, a metabolic pathway for dinoseb was developed and is shown in Figure III-1.

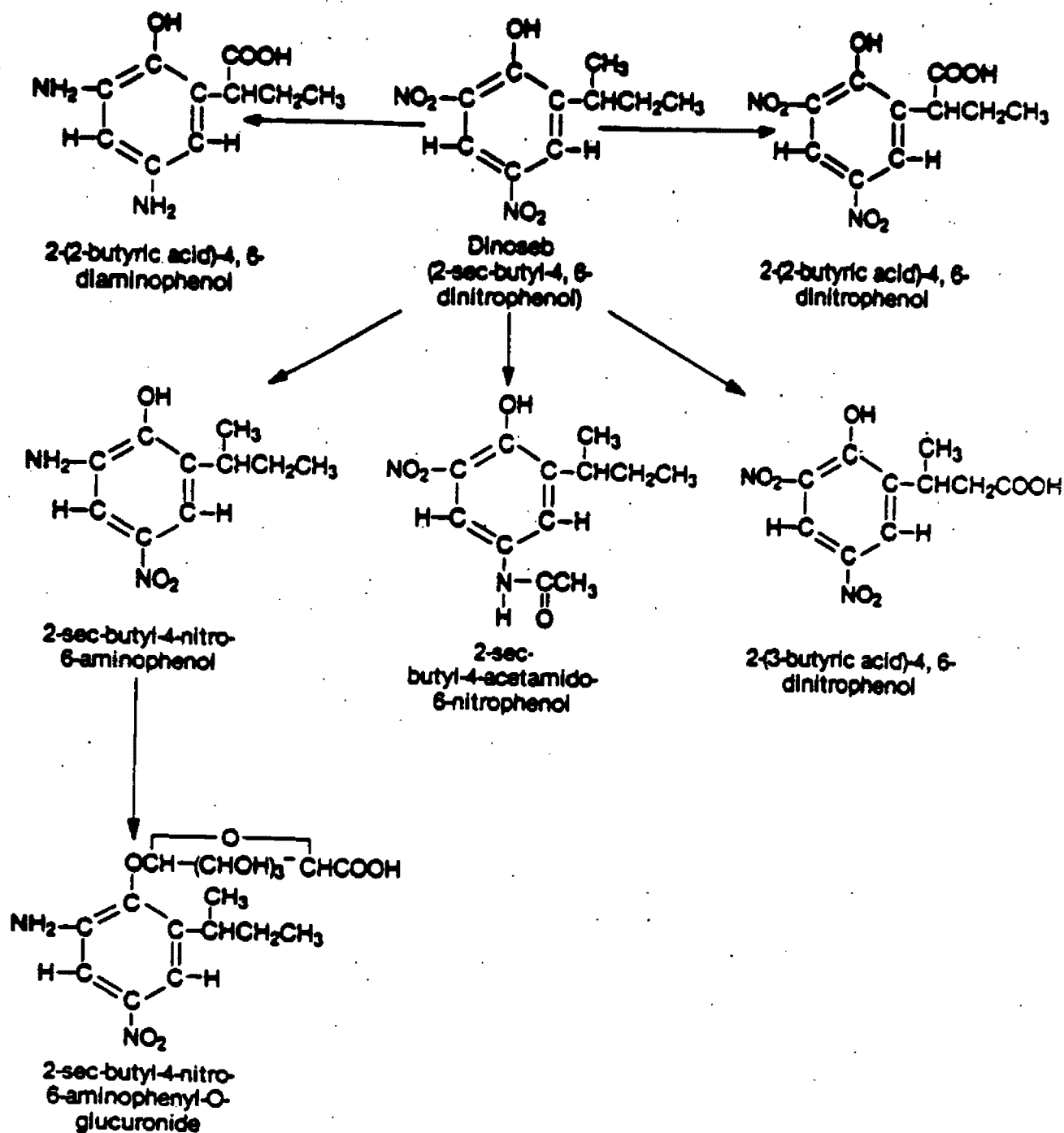


Figure III-1. Metabolic pathways of dinoseb in mammals based on reported metabolites.

SOURCE: Adapted from Ernst and Bar (1964); Froslic and Karlog (1970); Bandal and Casida (1972).

D. EXCRETION

Bandal and Casida (1972) administered 8 to 10 $\mu\text{mol/kg}$ (1.9 to 2.4 mg/kg) of ^{14}C ring-labeled DNBP (99% pure) by stomach tube to male albino rats (180 g) and mice (20 g) and measured urinary and fecal excretion over a 72-hour period. Animals were held in individual metabolism cages while receiving food and water ad libitum. Rats excreted approximately 65% of the radioactivity in the urine and 25% in the feces within 72 hours (a total of approximately 90% of the dose). Mice excreted 74% of the dose within 72 hours, with approximately equal quantities in the urine and the feces.

Gibson and Rao (1973) dosed nine nonpregnant female Swiss-Webster mice with 32 mg/kg (orally) or 17.7 mg/kg (intraperitoneally) of ^{14}C -labeled dinoseb. Urine and feces were collected over the subsequent 64 hours, and excretion was expressed as a cumulative percentage of the administered dose. In a comparable study, the common bile duct of treated female mice was cannulated, and bile was collected in tared scintillation vials. The bile was weighed, solubilized, and counted for radioactivity. The results (shown in Table III-3) indicated that in mice, orally ingested dinoseb is excreted in both urine (26%) and feces (30%), with relatively lower levels found in the bile (1.4% at 8 hours). Roughly similar values were observed in urine samples after intraperitoneal dosing, but higher levels were observed in feces (41%) and bile (10%).

Gibson and Rao (1973) measured the kinetics of clearance of ^{14}C -dinoseb from five female Swiss-Webster mice following a single oral dose of 32 mg/kg. Excretion was first order, with a rate constant of 0.02 hour^{-1} . This corresponds to a $t_{1/2}$ of 34.6 hours. Excretion was more rapid after a single intraperitoneal dose of 7.7 mg/kg (rate constant = 0.09 hours^{-1} , $t_{1/2}$ = 7.7 hours).

St. John et al. (1965) conducted a feeding experiment with four catheterized Holstein cows receiving dinoseb in the grain feed at 5 ppm for 3 days. No dinoseb residues were found in the milk. The levels of dinoseb in the urine on days 1 through 6 after dosing were, respectively, 0.17, 0.05,

Table III-3. Excretion of ¹⁴C-Dinoseb by Female Mice Following Oral or Intraperitoneal Administration

Time after administration (hr)	Mean cumulative excretion ^a					
	Bile		Urine		Feces	
	Oral (32 mg/kg)	ip (17.7 mg/kg)	Oral (32 mg/kg)	ip (17.7 mg/kg)	Oral (32 mg/kg)	ip (17.7 mg/kg)
0.5	0.1±0	0.2±0.1	-	-	-	-
1	0.4±0.1	0.6±0.1	0.8±0.2	1.4±0.2	-	-
2	0.6±0.3	1.4±0.4	1.9±0.4	3.9±0.1	-	-
4	0.9±0.4	3.9±0.6	3.2±0.4	7.0±0.1	-	-
8	1.4±0.6	9.6±1.4 ^b	6.8±1.4	13.4±1.3	0.5±0	3.3±0.9
16	- ^c	-	14.4±2.0	22.1±2.1	4.3±1.1	11.1±1.1 ^b
32	-	-	23.2±3.5	26.3±1.9	9.7±3.7	28.7±4.8 ^b
64	-	-	26.3±3.3	28.2±2.5	30.4±7.5	40.8±6.5

^aData are expressed as percentage of administered radioactivity. All values are means for groups of three mice ± SEM.

^bp < 0.05 (oral versus intraperitoneal).

^cNo data provided.

SOURCE: Adapted from Gibson and Rao (1973).

0.18, 0.14, 0.09, and 0.46 ppm. Urinary excretion on day 6 represented 3.5% of the administered intact compound as no conjugate formation was detected.

E. BIOACCUMULATION AND RETENTION

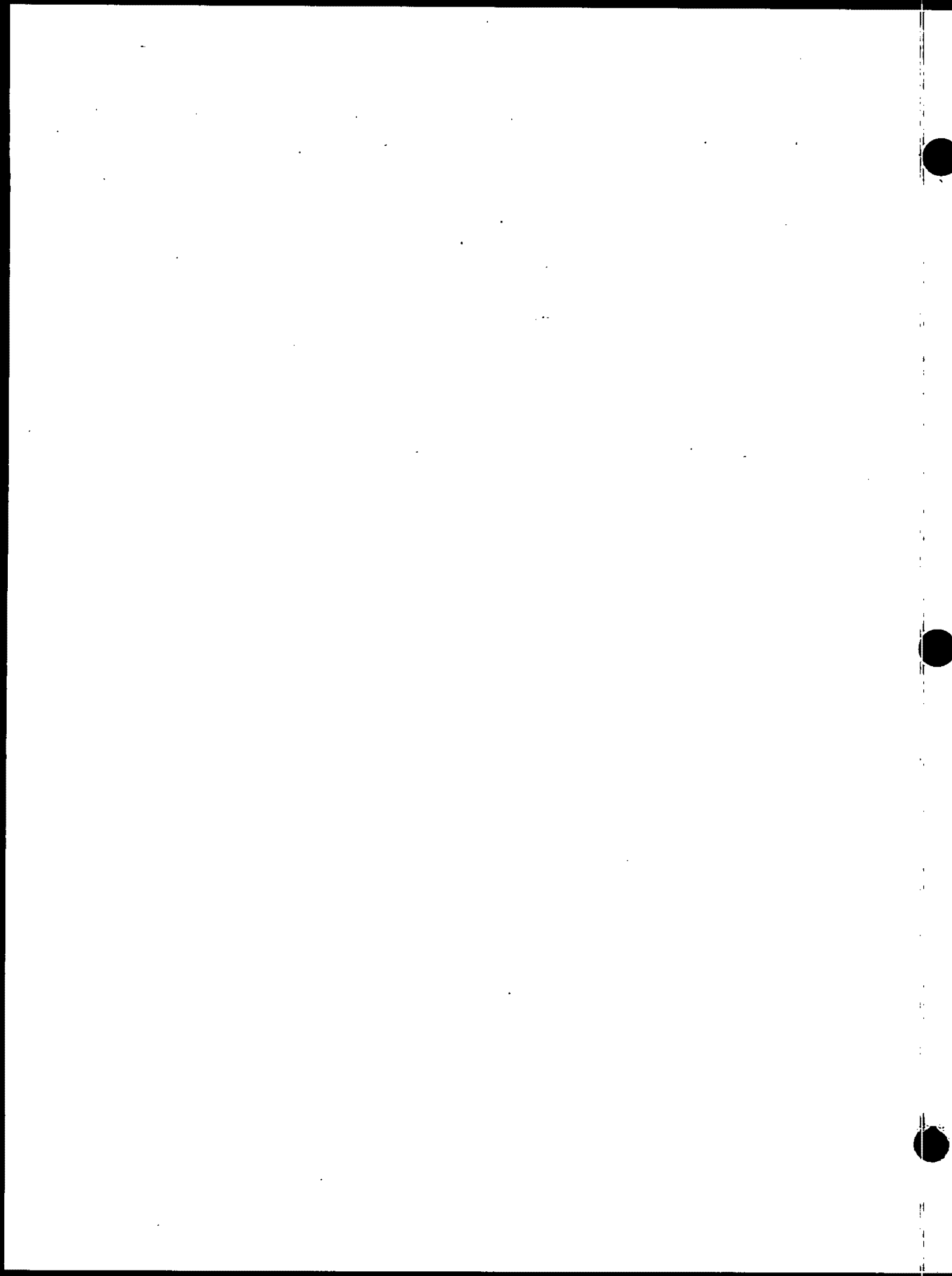
No studies were located that provided data on tissue or body levels of dinoseb following long-term oral exposure.

F. SUMMARY

About 25 to 37% of an oral dose of dinoseb is excreted in the feces. Approximately 40% of an intraperitoneal dose of dinoseb is excreted in the feces, suggesting that fecal excretion results primarily from secretion of absorbed dinoseb into the intestines via the bile. On this basis, absorption may be estimated to be essentially complete. A maximum of approximately 7% of the administered dose was found in the urine of monkeys following percutaneous administration of dinoseb. Absorbed dinoseb is distributed to all tissues of the mouse; however, brain and embryonic levels in pregnant mice never exceeded 2.5% of the maternal plasma level. Dinoseb is extensively metabolized by several pathways in animals: (1) one or both of the nitro groups can be reduced to the amine, which may then be acetylated; (2) the terminal methyl groups of the side chain can be oxidized to carboxyl groups; and (3) the compound or its metabolites may be conjugated, primarily as glucuronides. Although several metabolites have been identified, a greater number have been detected but not yet characterized. Dinoseb and its metabolites are excreted in both the urine and feces, with low amounts present in the bile. Excretion was monophasic following a single oral dose, with a $t_{1/2}$ of about 35 hours in mice. A $t_{1/2}$ of about 8 hours was observed following an intraperitoneal dose.

IV. HUMAN EXPOSURE

This section will be provided by the Science and Technology Branch, ODW.



V. HEALTH EFFECTS IN ANIMALS

A. SHORT-TERM EXPOSURE

1. Lethality

Acute oral lethality data for dinoseb are summarized in Table V-1. Estimates of oral LD_{50} values in mice, rats, guinea pigs, and rabbits range from 14 to 114 mg/kg. In a study by the Dow Chemical Company, it was reported that the oral LD_{50} of dinoseb (purity of 96.8%) in rats was 58.29 mg/kg (Industrial Biotest Laboratories, Inc., 1966).

Biggs et al. (1964), using dinoseb (purity of 18.5%) as a reference compound, determined the oral LD_{50} to be 39, 29, 26, and 50 mg/kg in rats, mice, guinea pigs, and rabbits, respectively.

The LD_{50} values in rats and mice were reported in two studies by the Dow Chemical Company (Mastri, 1970; Wazeter and Long, 1968). In the study with rats, doses of 23.41 to 79.01 mg/kg (purity of 96.8%) were administered orally to two animals of each sex per dose group, and the LD_{50} was determined to be 79 mg/kg. In the study with mice, doses of 14.7 to 68.1 mg/kg (purity not given) were administered orally to five male mice per dose group, and the LD_{50} was determined to be 41.4 mg/kg; 0/5 mice died at the 31.6-mg/kg dose level, and 4/5 mice died at the 46.4-mg/kg dose level. In additional studies by Rowe et al. (1966a), dinoseb (purity not given) was administered by intubation, and an LD_{50} of 40 mg/kg (32 to 50 mg/kg) was calculated for rats; 25 mg/kg (20 to 31 mg/kg) for guinea pigs; and 26 mg/kg (18 to 37 mg/kg) for chicks. The dose levels tested ranged from 5 to 60 mg/kg.

In a study conducted at Biochemical Research Laboratories, the LD_{50} in rats was reported to be 114 mg/kg (Rowe et al., 1966b). Doses tested were from 89 to 146 mg/kg (purity not given). In the same report, the LD_{50} is reported to be 88 mg/kg (80 to 97 mg/kg) for guinea pigs and 70 mg/kg for chicks (48 to 103 mg/kg). These values were higher than those of other researchers.

Table V-1. Acute Oral Toxicity of Dinoseb

Species	Sex	Dose (mg/kg)	LD ₅₀ (mg/kg) (LD or No. deaths/total tested)	Reference
Mouse	M	20-40	LD ₅₀	Bough et al. (1965)
Mouse	--*	--	29	Biggs et al. (1964)
Mouse	M	14-68	41	Wazeter and Long (1968)
Rat	M	25-40 35	LD ₅₀ LD	Bough et al. (1965) Ernst (1968)
Rat	M/F	60	LD	Spencer et al. (1948)
Rat	--	--	39	Biggs et al. (1964)
Rat	M/F	--	58	Industrial Biotest Laboratories Inc. (1966)
Rat	M	89-146	114	Rowe et al. (1966b)
Rat	M/F	23-79	58	Mastri (1970)
Rat	M/F	32-50	40	Rowe et al. (1966a)
Guinea pig	F	20-40	LD ₅₀	Bough et al. (1965)
Guinea pig	--	--	26	Biggs et al. (1964)
Guinea pig	M/F	20-31	25	Rowe et al. (1966a)
Guinea pig	M/F	80-97	88	Rowe et al. (1966b)
Rabbit	--	--	50	Biggs et al. (1964)
Sheep	M/F	45	2/4	Froslie (1976)
Cattle	F	15 g total	0/1	Froslie and Karlog (1970)
Chick	M	40-80	LD ₅₀	Bough et al. (1965)
Chick	M/F	18-37	26	Rowe et al. (1966a)
Chick	M/F	48-103	70	Rowe et al. (1966b)

*Data not provided.

Bough et al. (1965) reported on the acute oral toxicity of dinoseb (purity 99%) in several animal species. Symptoms of poisoning in guinea pigs included prostration, rapid respiration, and convulsions immediately preceding death. Blood levels of DNBP in 10 female guinea pigs receiving 40 mg/kg increased from 0 mg/100 mL to about 8.4 mg/100 mL at the time of death, even though death occurred from 1.7 to 3.0 hours after dosing. Spencer et al. (1948) conducted acute oral toxicity studies of DNBP (purity of 99.1%) in rats and reported that death occurred 1 or 2 hours after feeding or not at all. The authors suggested that deaths were due to the pyretic effects of the chemical. The authors report the "survival dose" (the largest dose that all animals survived) as 5 mg/kg and the "lethal dose" (the smallest dose causing death of all animals) as 60 mg/kg.

Palmer (1964) studied the toxicity of an alkanolamine salt of dinoseb (purity not given) in 1- to 2-year-old Delaine-merino sheep, sex not specified. One sheep fed two daily doses of 100 mg dinoseb as the alkanolamine salt died without preliminary signs of toxicity. A second animal received four daily doses of 50 mg/kg/day by gavage and was also found dead without premonitory signs. Necropsy findings included gastroenteritis, nephritis, hepatitis, evidence of anemia (indicated by an enlarged spleen), areas of hemorrhage, and edema of the heart.

The effect of ambient temperature on lethality was studied by Preache and Gibson (1975b). Swiss-Webster female mice administered dinoseb (purity not given) intraperitoneally were maintained at a high environmental temperature (32°C) for 24 hours or at a low temperature (0 to 6°C) for 1.5 to 4 hours. The LD₅₀ at 32°C was 20.2 as compared to 14.1 mg/kg for the mice maintained at 0 to 6°C for 1.5 hours. The LD₅₀ values after 1.5 and 4 hours at 0 to 6°C were comparable.

The acute intravenous LD₅₀ values for dinoseb in rats and mice were reported to be 8 and 9 mg/kg, respectively (Biggs et al., 1964).

Acute dermal lethality data for dinoseb are summarized in Table V-2. Estimates of dermal LD₅₀ values in rats range from 67 to 134 mg/kg. Roughly

Table V-2. Acute Dermal Toxicity of Dinoseb

Species	Sex	Dose (mg/kg)	Application site	LD ₅₀ or No. deaths/ total tested	Reference
Mouse	M	100	Abdomen	2/10	Bough et al. (1965)
	M	500	Abdomen	9/10	Bough et al. (1965)
Rat	M	67-134	Back	LD ₅₀	Noakes and Sanderson (1969)
Guinea pig	-	100	Abdomen	0/5	Spencer et al. (1948)
	-	150	Abdomen	1/5	Spencer et al. (1948)
	-	200	Abdomen	4/5	Spencer et al. (1948)
	-	300	Abdomen	5/5	Spencer et al. (1948)
Rabbit	-	-*	Abdomen	3/3	Spencer et al. (1948)
	-	10	Back	0/4	Bough et al. (1965)
	-	20	Back	4/4	Bough et al. (1965)
	-	40	Back	4/4	Bough et al. (1965)
Rabbit	-	50	Skin	2/2	Wolf (1959)
	-	20	Skin	0/2	Wolf (1959)
	-	10	Skin	0/2	Wolf (1959)

*Death occurred following three to eight applications of 3% alcoholic solution of dinoseb. Total dose not specified.

similar results for the dermal toxicity of dinoseb have been reported in mice and guinea pigs, but rabbits may be more sensitive to the chemical (Spencer et al., 1948; Bough et al., 1965; Noakes and Sanderson, 1969). In a study by the Dow Chemical Company, the acute dermal LD₅₀ for guinea pigs was reported to be in the range of 100 to 500 mg/kg dinoseb (purity of 99.1 to 99.7%). The survival dose was 100 mg/kg. No other information was provided.

2. Other Acute Effects

Dandliker et al. (1980) reported the effect of a single oral dose of dinoseb (about 20 mg/kg) (purity not given), administered by intubation, on the immune response of inbred male hamsters, strain LHC/LAK, age 5 to 8 weeks and weighing about 100 g. The dose was described as one-half the LD₅₀ reported by Thompson (1976), which was 37 to 50 mg/kg. Dinoseb markedly depressed the cellular immune response as measured by two methods: (1) visual elevation of the intensity of inflammation and swelling of an antigen-injected footpad as compared with the contralateral footpad treated with buffer alone; and (2) differential temperature measurement between the antigen-injected footpad and the control footpad. Dinoseb also depressed the humoral immune response, as measured by fluorescence polarization measurements after adding fluorescein to an Ig preparation from serum. The authors point out that the actions of dinoseb were remarkably long lasting (remaining 49 days after dosing) and suggest that the effect is probably the result of a decreased level of antibodies rather than of any change in the type of antibodies produced.

Froslic and Karlog (1970) reported that a single 15-g dose of DNBP (purity not given) administered into the rumen of two cows produced toxic effects, including increased pulse rate and total anorexia. A reddish-brown urine was observed. At 2 to 4 hours after ingesting the herbicide, the cattle had methemoglobin blood levels of 30 to 40%. Within 2 weeks, however, the animals appeared to recover. A methemoglobin concentration of 10% could still be measured after 10 days, and hemolysis persisted for several days.

Froslic (1974) reported a study of acute DNBP (purity not given) toxicity in sheep. Seven sheep received a single dose at 45 mg/kg administered by tube into the rumen. The dinoseb was dissolved in approximately 200 mL of a 33% solution of acetone in water. All animals showed hemolysis of red blood cells and methemoglobinemia formation. The acute phase of toxicity (lasting from 6 to 8 hours) was characterized by dyspnea, hyperthermia, methemoglobinemia, and hemoconcentration. After 1 to 2 days, hemoglobinemia and hemoglobinuria were the predominating clinical signs. The methemoglobinemia lasted for 2 to 3 days with maximum methemoglobin values of 5 to 8 g/100 mL. Liver and kidney dysfunction and a significant reduction in plasma proteins occurred during this acute phase. Glutathione levels in the red blood cells were also markedly decreased. Postmortem lesions consisted of moderate congestion and degenerative changes in the liver and kidney. One animal showed the discoloration of methemoglobinemia, while other animals that died during a hemolytic crisis were icteric, with extensive degenerative changes in the liver and kidneys. Two animals died and one was euthanized during the first 4 days of the study; all others survived. The author concluded that the effects of DNBP include both an initial and a delayed phase. The initial effect is partly related to the unmetabolized dinitrophenol, which produces dyspnea and hyperthermia. A major factor in this stage, however, is the formation of diamino metabolites in the rumen, which results in methemoglobinemia, hypoproteinemia, and hemoconcentration. According to the author, the diamino metabolites also contribute to the later stages of poisoning, which include lysis of red blood cells.

Spencer et al. (1948) investigated the potential of dinoseb (purity not given) to produce cataracts in White Pekin ducklings. The experiment was based on the knowledge that 2,4-dinitrophenol produces cataracts in humans and that ducklings and chicks appear to be suitable laboratory animals for such studies. Ducklings that received 0.25% dinoseb in the diet died within 3 days but had no cataracts. Animals receiving 0.1% in the diet died within 4 days, with one animal showing cataracts. Half of the animals ingesting dietary levels of 0.03% died within 5 days. At this exposure, cataracts were observed in one duckling on the fifth day and in another on the eighth day, at which

time the birds were accidentally killed. This study demonstrates the ability of dinoseb to produce cataracts in experimental animals.

Spencer et al. (1948) reported that of 10 albino rats (Breeding and Laboratory Institute, Brooklyn) fed a diet delivering dinoseb at 13.5 mg/kg/day (99.1%), 4 died between days 5 and 13. The remaining six animals were sacrificed on day 21. They showed marked emaciation, an empty gastrointestinal tract, and a blood urea nitrogen of 55 mg% (controls: 19.4 mg%). Microscopic examination of tissues revealed slight degenerative changes in the renal tubules and slight cloudy swelling of the liver, but no appreciable changes in lung, heart, spleen, adrenal, pancreas, or testes.

Pawlowski (1970) studied the central nervous system (CNS) effects of dinoseb (purity not given) in the Wistar rat. The animals were dosed intraperitoneally once each day for 5 days. Dose levels of 2 and 8 mg/kg/day were without effect. Doses of 12 or 16 mg/kg/day intensified inhibitory and excitatory activities in the brain, resulting in shorter periods of time needed for the development of escape reaction, increased infrequency of positive conditioned reactions, and more efficient differentiation between stimuli. Doses of 20 or 24 mg/kg/day provoked the inhibition of conditioned reflex activity.

B. LONG-TERM EXPOSURE

1. Subchronic Effects

Hall et al. (1978) reported on the toxic effects of dinoseb (purity of 80%) administered in the diet to 35- to 38-day-old Sherman rats for 60 days. Groups of 14 rats of each sex were fed a diet fortified with technical grade dinoseb at 0, 50, 100, 150, 200, 300, 400, or 500 ppm. All animals receiving dietary levels of 400 and 500 ppm died within 3 weeks. Of the animals receiving 300 ppm, 14% died within 21 days, and this group was not continued on the diet. Growth was depressed at all lower dietary levels (50 to 200 ppm). Organ weights (liver, spleen, heart, lung, brain) decreased, and

organ-to-body weight ratios increased. Blood alkaline phosphatase and alanine aminotransferase activities and potassium and urea nitrogen contents were significantly increased, and lactic dehydrogenase and cholinesterase activities were decreased. Tissue residue levels were dose related in the following order: blood>feces>urine>adipose>brain>liver. Discrimination learning was not affected, and locomotor activity was increased at 200 ppm. Diffuse tubular atrophy of the testes was observed, particularly in animals receiving 200 ppm. Assuming that 1 ppm in the diet equates to a dose of approximately 0.05 mg/kg/day, the dietary level of 50 ppm equals 2.5 mg/kg/day. Based upon the organ-to-body weights and hematological changes, no NOAEL was obtained, and 2.5 mg/kg/day was established as a LOAEL.

Spencer et al. (1948) conducted a 6-month dietary study with white male rats (Breeding and Laboratory Institute, Brooklyn) fed dinoseb (99.1% pure) mixed in the diet. Food and water were available ad libitum. Thirty rats served as controls; three groups of 20 rats were administered dinoseb at dietary levels of 1, 3.5, 2.7, or 5.4 mg/kg/day; and 10 rats received 13.5 mg/kg/day. Nonpalatability was observed, although the actual food intake was not reported. Four rats that received 13.5 mg/kg/day died within 13 days; the six survivors appeared markedly emaciated and were sacrificed on day 21. The body weights of the animals receiving 5.4 mg/kg/day were 3 to 8% lower than those of the control animals during the 6-month study period ($p < 0.05$). No other discernible toxic effects were noted in these animals during the study. No effects on erythrocyte count, hemoglobin concentration, leukocyte count, and differential count were observed. The average blood urea nitrogen was 20.3 mg/100 mL compared with 17.5 mg/100 mL for the controls. Organ weights were comparable to those of controls, except for an increase in liver weight ($p < 0.01$) in rats fed 5.4 mg/kg/day of dinoseb. Gross and microscopic examination of tissues failed to reveal any appreciable changes. Animals at the two lower dosage levels (2.7 or 1.35 mg/kg/day) had growth curves that were comparable to those of the controls; blood urea nitrogen levels, organ weights, and histopathology were also similar to the control group. Thus, 2.7 mg/kg/day represents a NOAEL for 6-month dietary exposure in rats.

In a study conducted by McCollister et al. (1967), groups of four male and four female beagle dogs per dose were fed diets containing 0.005, 0.01, 0.02, or 0.03% dinoseb (purity of 97.5%) for 91 days. Dogs receiving 0.005 or 0.01% did not exhibit adverse effects. Females receiving 0.02 and 0.03% showed slight growth retardation, increased average weights of liver, mural endocarditis, and microscopic heart changes. Dogs removed from these test diets after 91 days and given half their basic ration for an additional 37 days gained weight, and their average liver-to-body weight ratios increased; no histopathological changes were seen. The NOAEL was established as 4 mg/kg/day, based upon the lack of effects at 0.01% (100 ppm). The dose conversion was based on food consumption data.

2. Chronic Effects

Two chronic toxicity studies have been reported, one with rats and the other with mice. Hazleton (1977) conducted a 2-year feeding study with groups of 60 albino rats/sex (Charles River CD) at dose levels of 0, 1, 3, and 10 mg/kg/day (purity not given). Hunched appearance and staining of the fur were noted more often in the dosed animals when compared to controls. Polypnea was noted in all treated animals, particularly females, during the first year of the study. Mean body weight gains of males receiving the mid and high doses and females receiving all doses were slightly to moderately lower than those of controls during the first year of the study; the decrease was statistically significant. At study termination, the mean body weights were still lower than those of controls, but the decreases were not statistically significant. There were no compound-related effects on survival, food consumption, hematology, clinical chemistry, and urinalysis. Palpable nodules and tissue masses were first noted by week 34 and were more frequently seen in females than males. Gross pathology showed lung abnormalities and liver discolorations in dosed and control animals. There were no effects on mean organ weights between control and dosed animals. However, a significant ($p = 0.05$) decrease in mean thyroid weight was observed at all dose levels in male rats. A dose-related trend in decreased thyroid weights was also observed. No histopathological changes were detected, but tissues for only

10 animals per sex from the control and high-dose groups and the liver, kidneys, and lesions from the low- and mid-dose rats were examined at interim sacrifice on week 52 and at final sacrifice on week 104. Based on decreased thyroid weights, a NOAEL was not established for this study, and 1 mg/kg/day was designated as a LOAEL.

In a chronic feeding study with mice, groups of 70 male and 70 female CD-1 mice were administered 0, 1, 3, and 10 mg/kg/day of technical grade dinoseb (purity of 98%) in the diet for 100 weeks (Brown, 1981). Beginning in week 10 of the study and lasting throughout, body weight gain was reduced in the mid- and high-dose females only. Low-dose females and all males were unaffected. Food consumption, hematology, and urinalysis did not reveal any treatment-related changes. A small number of high-dose males showed very high fluctuations in plasma alkaline phosphatase, while most of the animals in the group were within normal range. The group differences were not statistically significant. Lenticular opacities were observed at the 3- and 10-mg/kg/day dose levels, but animals receiving the low dose were not examined. Cystic endometrial hyperplasia and atrophy were observed in females, and hypospermatogenesis and degeneration were seen in the testes of males receiving 1, 3, and 10 mg/kg/day. Thus, no NOAEL was identified, and the LOAEL for this study was 1 mg/kg/day.

C. DEVELOPMENTAL/REPRODUCTIVE EFFECTS

1. Developmental Effects

a. Oral

Gibson (1973) reported on the teratogenic effects of dinoseb (purity not given) in Swiss-Webster mice. The compound was administered, by gastric intubation, at doses of 20, 32, or 50 mg/kg/day in aqueous solution to groups of pregnant Swiss-Webster mice on days 8 to 16, 10 to 12, or 14 to 16 of gestation. Significant increases of supernumerary ribs were found when 20 mg/kg dinoseb was administered throughout organogenesis (8 to 16 days). At

32 mg/kg/day, a slight, but significant, reduction in fetal crown-rump distance (2.4 cm compared with 2.6 cm in controls) was observed along with a significant increase of supernumerary ribs and vertebrae, and absent or unossified sternbrae. Doses of 50 mg/kg killed approximately 75% of the dams but produced no effects on fetal survival or size. Skeletal anomalies were not observed; however, only two litters were available for examination owing to maternal toxicity. The author concluded that the NOAEL for dinoseb administered orally during organogenesis was 20 mg/kg/day, even though there was a statistically significant increase in the incidence of supernumerary ribs. It was pointed out that the positive findings were present only at dose levels associated with some maternal lethality.

Chernoff and Kavlock (1983) studied the maternal and perinatal effects of dinoseb (purity not given) administered orally at 15 mg/kg/day to pregnant CD-1 mice. The chemical was given on gestation days 8 through 12. No effect was observed on maternal weight, number of surviving pups, or pup weights 1 and 3 days postpartum.

In another study, Kavlock et al. (1985) assessed the effects of acute maternal toxicity upon fetal development. Pregnant CD-1 mice were placed in groups of 15, 20, and 40, and orally administered single doses of dinoseb (purity of 97%) at 0, 26, and 33 mg/kg, respectively, on day 8 of gestation. The animals were sacrificed on day 18 of gestation, and the uteruses were removed and weighed. The fetuses were removed from the uterus, weighed, and examined for any gross malformations. The only significant fetal effect was an increase in the incidence of supernumerary lumbar ribs. This effect was inversely related to maternal weight gain. Other isolated malformations include encephalocele, exencephaly, miscellaneous cranial defects, and an umbilical hernia.

Spencer and Sing (1982) reported the effect of dinoseb (purity of 95%) on pregnant Sprague-Dawley rats. Dinoseb was added to the diet from days 6 through 15 of gestation. Decreased maternal body weights, ataxia, lethargy, decreased levels of placental protein and glycogen, and a significant

reduction in embryo survival were found at doses of 8.60, 9.38, 9.49, and 10.86 mg/kg. Consumption of 9.23 mg/kg dinoseb resulted in decreased maternal weight and a significant decrease in pup survival at birth. A dose of 6.9 mg/kg/day did not result in maternal weight loss or other toxic signs; however, there was a nonsignificant decrease in fetal survival. At higher doses, reduction in fetal survival reached statistical significance. The effect of dinoseb on pregnancy performance is shown in Table V-3. In this study, the NOAEL was found to be 3.26 mg/kg/day, and the LOAEL was found to be 6.9 mg/kg/day.

In a study with pregnant Wistar rats, dinoseb (purity of 96.1%) was administered by gavage to groups of 25 mated rats at doses of 0, 1, 3, or 10 mg/kg/day on days 6 through 15 of gestation (Becker, 1986a). Decreased total body weight gains (not significant) were noted in dams receiving 10 mg/kg/day. Reduced ossification corresponded with the significantly reduced fetal body weights ($p < 0.05$) at the high dose and were considered to be an effect of the maternal toxicity. There was an increase in the incidence of bilateral supernumerary ribs at the high-dose level. The developmental NOAEL was identified as 3 mg/kg/day.

Becker (1986b) administered dinoseb (purity of 98.1%), by gavage, to groups of 16 mated chinchilla rabbits at doses of 0, 1, 3, or 10 mg/kg/day from days 6 through 18 of gestation. No maternal toxicity was noted in any group. Increased incidences of external, visceral, and skeletal malformations seen in the high-dose group were considered to be compound-related and included anomalies in 40 fetuses (32.8%) in 11 of 16 litters. Multiple anomalies were noted in 26 fetuses (21.3%). Compound-related malformations were predominantly microphthalmia, anophthalmia, and internal hydrocephaly. Neural tube defects such as dyscrania associated with hydrocephaly, scoliosis, kyphosis, dysmorphogenesis of caudal and sacral vertebrae, and encephalocele were noted in approximately 31% of the litters from dams receiving 10 mg/kg/day. Dinoseb was considered to be teratogenic at levels of 10 mg/kg/day. The developmental NOAEL and LOAEL were 3 and 10 mg/kg/day, respectively. The maternal NOAEL was 10 mg/kg/day.

Table V-3. Effect of Dinoseb on Pregnancy Performance in Rats

Dietary treatment ^a (ppm)	Intake of dinoseb (mg/kg/day)	Number of litters	Implantations at day 6 per dam	Number of conceptions at day 12	Percent embryo survival per litter at day 12 ^b	Percent pup survival per litter at birth ^c	Fetal birth weight per litter (g)
0	--	6	12.5 ± 1.4 ^d	12.5 ± 1.4	100 ± 00.0	80.12 ± 7.59	7.20 ± 0.30
50	3.26 ± 0.09	6	11.7 ± 1.9	11.7 ± 1.9	100 ± 00.0	83.31 ± 12.56	7.13 ± 0.27
100	6.90 ± 0.20	6	13.7 ± 1.1	13.7 ± 1.1	100 ± 00.0	63.09 ± 6.05	6.78 ± 0.14
150	9.23 ± 1.02	6	14.3 ± 0.7	14.3 ± 0.7	100 ± 00.0	45.9 ± 11.56 ^e	--
200	10.86 ± 1.33	6	13.2 ± 1.4	9.7 ± 1.1	75 ± 9.0 ^e	53.06 ± 9.20 ^e	6.43 ± 0.18 ^e
250	9.38 ± 2.05	6	12.3 ± 0.7	7.3 ± 2.6	56 ± 22.3 ^e	16.34 ± 12.32 ^e	--
300	9.49 ± 1.46	6	14.8 ± 2.1	4.9 ± 3.5	33 ± 21.0 ^e	10.81 ± 5.56 ^e	--
350	8.60 ± 1.57	6	12.2 ± 0.4	0.0 ± 0.0	00 ± 00.0	0.00 ± 0.00	--

^aDinoseb administered from day 6 through 15 of pregnancy.

^bPercent embryo survival: the ratio of the number of surviving embryos per litter at day 12 to that at day 6, as expressed in percentage.

^cPercent pup survival: the ratio of the number of live pups at birth to the number of implantation sites counted at day 6, as expressed in percentage.

^dAll results expressed as mean ± SE.

^eSignificantly different from the control, using Student's test (p < 0.05).

SOURCE: Adapted from Spencer and Sing (1982).

Gray and Kavlock (1984) orally dosed pregnant CD-1 mice with dinoseb (purity of 97%) and determined development. Pregnant CD-1 mice were administered oral doses of dinoseb at levels of 0 and 5 mg/kg daily during days 8 to 12 of gestation. At that time, the pups were weighed, counted, and, along with the dams, randomly separated and housed in litters of six animals. At 30 days of age, the pups were weaned, counted, weighed, and housed for breeding purposes. During the 250-day study period, the mice were observed for any changes or lingering neonatal effects. Pups born during the study were counted and the length of gestation recorded. At 250 days of age, the males were sacrificed and necropsied. No significant effects on pup survival and weight, weaning survival and weight, viability, body and organ weight, or gross pathology were noted.

b. Intraperitoneal

Gibson (1973) also administered intraperitoneal doses (purity not given) of 0, 10, 12.5, 15.8, 17.7, 18.8, and 20.0 mg/kg/day to Swiss-Webster mice on gestation days 10 to 12. Additional mice received 0, 12.5, or 17.7 mg/kg/day on days 14 to 16 or 5 mg/kg/day on days 8 to 16. Doses of 15.8 mg/kg or below were not maternally toxic, whereas hyperthermia and some lethality were noted at 17.7 and 18.8 mg/kg. None of the dams survived at 20 mg/kg. Subtoxic doses of 10 to 15.8 mg/kg had no developmental effects when given during days 10 to 12; however, increased resorption rates and reduced fetal weights were significant for dams given 12.5 mg/kg on days 14 to 16. Fetuses from dams dosed with 17.7 mg/kg/day on days 10 to 12 of gestation had reduced weights and a variety of gross anomalies (oligodactyly, imperforate anus, acaudia, microcaudia, and amelia), soft tissue anomalies (internal hydrocephalus, hydronephrosis), and skeletal anomalies (fused ribs, missing ribs, fused and missing sternebrae, fused, unossified, or absent vertebrae, and absent or unossified long bones). Fetuses from the 18.8-mg/kg group had significantly reduced weights and lengths and increased incidences of fused vertebrae and fused ribs when compared to controls. An intraperitoneal dose of 5 mg/kg/day given throughout organogenesis (days 8 to 16) produced no developmental effects.

Preache and Gibson (1975b) studied the effect of drugs that alter hepatic drug-metabolizing activity on the fetal toxicity of dinoseb (purity not given). Swiss-Webster mice were treated intraperitoneally with dinoseb at doses of 0, 14.1, or 15.8 mg/kg/day on days 10 to 12 of gestation. Subgroups of each set of dosed mice were deprived of food for 0, 24, or 48 hours from the ninth day of gestation. In a second study, two groups of pregnant mice were given single injections of either 17.7 mg/kg on day 11 of gestation or 18.8 mg/kg on day 12 of gestation. For approximately half of the mice in each group of the second study, dinoseb administration was preceded by treatment with 50 mg/kg phenobarbital twice each day for 3 days; the remaining mice were not pretreated. Two other groups received 15.8 or 17.7 mg/kg dinoseb 1 hour after treatment with 32 mg/kg of SKF-525A on day 12 of gestation. A third group served as the untreated control. On the 19th day of gestation, fetuses were removed by cesarean section and were weighed and examined for external anomalies. Half of the fetuses of each litter were fixed in Bouin's solution and examined for soft tissue anomalies. The remaining fetuses were stained with alizarin red S and examined for skeletal defects.

The results of this study indicated that SKF-525A potentiated and phenobarbital inhibited the resorptions and reductions in fetal body weight induced by dinoseb. Dinoseb-induced external, soft tissue, and skeletal anomalies were increased by 24-hour food deprivation and SKF-525A pretreatment, and 48-hour food deprivation had minimal adverse effects on fetal weight and ossification of small bones. In general, the action of phenobarbital protected against dinoseb teratogenicity. Disposition of radiolabeled dinoseb (15.8 mg/kg) was also examined in adult female mice following each pretreatment. Food deprivation for 24 hours slowed and phenobarbital pretreatment hastened the disappearance of dinoseb from the plasma. Food deprivation for 48 hours and SKF-525A pretreatment did not affect the disappearance of dinoseb from the plasma but did increase and decrease, respectively, its disappearance from the liver. The authors suggested that the alterations in dinoseb-induced embryotoxicity and teratogenicity produced by the pretreatments were related

to an alteration in the rate of oxidative metabolism and clearance of dinoseb from the mother (Preache and Gibson, 1975b).

McCormack et al. (1980) reported a study of postnatal morphology and functional capacity of the kidney in neonates of Sprague-Dawley rats injected intraperitoneally with dinoseb (purity not given) at doses of 0, 6.3, 8.0, 9.0, 11.2, 12.5, or 15.8 mg/kg/day on days 10 to 12 of gestation. At 21 days, some fetuses were removed by cesarean section and examined, and samples were selected for histological examination. Histological examination was also performed on selected tissues from offspring at day 1 or 42 postpartum. Renal function of rats exposed to dinoseb prenatally was determined both in vivo and in vitro. Transport capacity in the kidney was determined by measuring the ability of tissue slices to accumulate p-aminohippuric acid or N-methyl-nicotinamide. Postnatal renal function was also assessed in the 42-day-old rats by measuring inulin and p-aminohippuric acid clearance, blood urea nitrogen, and maximal urine osmolarity. Pregnant rats administered dinoseb at 11.2, 12.5, or 15.8 mg/kg/day all died within 1 week of treatment. At the dose of 9.0 mg/kg, the mortality rate of pregnant rats was 20%. At 8.0 mg/kg or less, no deaths occurred. Fetal weight was decreased by dinoseb at doses of 8.0 or 9.0 mg/kg/day. Fetal length was also reduced at the dose of 9.0 mg/kg. Postpartum studies indicated that pups exposed in utero weighed less than controls at days 1 and 7 postpartum but were not different from controls at 42 days of age. Livers from near-term fetuses of mothers treated with 8.0 to 9.0 mg/kg dinoseb on days 10 to 12 had many vacuolated cells. This effect persisted even through 42 days postpartum, along with the presence of necrotic cells. The nucleus was absent from soma cells and was pyknotic or karyorrhectic in others.

Kidney-to-body weight ratios were not affected by dinoseb treatment. However, approximately 40% of the near-term fetuses from dams treated with 8 or 9 mg/kg/day had dilated renal pelvises and/or ureters when examined grossly. Histological examination revealed dilation of renal pelvises and tubules. The transitional epithelium was vacuolated in ureters from near-term and 1-day-old rats treated with dinoseb in utero. The incidence and severity of kidney

lesions decreased with age of offspring. At 42 days postpartum, gross examination of kidneys and ureters did not reveal any dilated ureters, and only 3 of 28 animals at the prenatal dose of 9 mg/kg/day had dilated renal pelves. No microscopic differences were noted between ureters and kidneys of dinoseb-treated and untreated rats at 42 days of age. Other renal parameters measured in offspring were not affected by dinoseb treatment. In this study, the NOAEL for the pregnant females exposed on days 10 to 12 of gestation was 6.4 mg/kg. The authors suggest that the toxicity of dinoseb in pregnant female mice is greater than that in rats.

c. Subcutaneous

In addition to the oral and intraperitoneal studies, Gibson (1973) showed that subcutaneous injections of 17.7 mg/kg/day (purity not given) during late organogenesis (days 14 to 16 of gestation) or throughout organogenesis (days 8 to 16) produced overt maternal toxicity and decreased fetal survival and size in mice. Statistically significant gross or soft tissue anomalies were produced with doses of 17.7 mg/kg/day on days 14 to 16. An increased incidence of skeletal anomalies occurred in the offspring of dams treated with 17.7 mg/kg/day on days 10 to 12 and 8 to 16, but not during late organogenesis (days 14 to 16). A subcutaneous dose of 10 mg/kg/day revealed no effect at any of the dosing periods.

d. Dermal

In a dermal developmental toxicity study conducted by Argus Research Laboratories, Inc. (Hoberman, 1987), groups of 17 artificially inseminated New Zealand White rabbits were percutaneously administered dinoseb (99% purity) at doses of 0 (sham control), 1, 3, or 9 mg/kg/day on days 7 through 19 of gestation. In addition, 12 artificially inseminated rabbits were percutaneously dosed with 18 mg/kg/day. These doses were based on a dermal range-finding study in which nonpregnant female rabbits were given doses of 10, 25, 50, or 75 mg/kg daily for 3 days. After 2 days of dosing, however, a mortality rate greater than 10% occurred in groups receiving 9 or 18 mg/kg/day.

The dose level of remaining high-dose animals was then reduced to 9 mg/kg/day, and four untreated animals were reassigned to the 9-mg/kg/day group and received this dose level for the entire 13-day dosing period.

The animals were fitted with Elizabethan collars and exposed to dinoseb for approximately 6 hours a day, after which test sites were rinsed with an isopropanol water solution and blotted dry to remove the test material. The skin at the application site was evaluated daily, prior to dosing, using the Draize method to grade erythema, edema, and possible eschar formation. Surviving animals were delivered by cesarean section on day 29 of gestation. All dams, including those that died prior to study termination, animals that aborted, and sacrificed animals, were examined grossly, and a teratological evaluation of their offspring was conducted. Visceral and skeletal examinations were conducted on each fetus, and brains were examined using a single cut at the level of the anterior fontanelle.

As previously stated, a significant increase ($p \leq 0.01$) in mortality was noted in the high-dose groups. Mortality rates of 71% (15 of 21 animals) and 88% (7 of 8 animals) occurred in rabbits receiving 9 and 9(18) mg/kg/day of dinoseb. The author also considered the slight increase in mortality at 3 mg/kg (3 of 17 animals) to be due to dinoseb administration. Administration of dinoseb resulted in slight to moderate dermal irritation. Measurement of daily rectal temperatures indicated that body temperatures were elevated in animals receiving dosages of dinoseb at 3 mg/kg/day or higher. Total body weight gains for rabbits receiving dinoseb were slightly increased compared to those of controls (Table V-4). However, during the period of dose administration (gestational days 7 through 19), body weight gains were decreased for all groups, although the dinoseb-treated animals appeared to be more severely affected. These body weight decreases were probably due in part to the wearing of Elizabethan collars.

Gross observations of dams given 9 and 9(18) mg/kg/day attributable to dinoseb administration included the appearance of yellow musculature and

Table V-4. Summary of Body Weight Changes in Pregnant Rabbits Percutaneously Treated With Dinoseb During Days 7 to 19 of Gestation

Dosage level (mg/kg/day)	Body weights (kg) at gestational day:				Total weight gain
	0	7	19	29	
0	3.74 ± 0.37 ^a	3.85 ± 0.38	3.73 ± 0.45	3.80 ± 0.44	0.06
1	3.81 ± 0.33	3.95 ± 0.33	3.70 ± 0.42	3.90 ± 0.32	0.09
3	3.70 ± 0.31	3.83 ± 0.31	3.57 ± 0.46	3.82 ± 0.42	0.12
9	3.74 ± 0.38	3.88 ± 0.28	3.68 ± 0.33	4.00 ± 0.23	0.26
9(18)	3.51 ± 0.32	3.64 ± 0.36	4.03 ± 0.00	4.20 ± 0.00	0.69

^aMean ± SD.

SOURCE: Hoberman (1987).

subdermal tissue, and hemorrhaging of the brain, trachea, thymus, lungs, and subdermis of the thorax and abdomen.

Reproductive parameters such as numbers of implantations, corpora lutea, live fetuses per litter, and fetal body weights and sex ratios were comparable between control and dosage groups. Mean number of resorptions appeared to be increased (not statistically significant), and live litter size was decreased for the group receiving 9 mg/kg/day. A summary of reproductive parameters is presented in Table V-5.

Dose-related increases were observed in gross external, soft tissue, and skeletal malformations in offspring of dams given dinoseb at levels of 3 mg/kg or higher. Malformations attributed to dinoseb administration in the 3-, 9-, and 9(18)-mg/kg/day dosage groups included hydrocephaly, ectopic eye bulge, microphthalmia, anophthalmia, craniosynostosis (includes all malformations related to fusion or incomplete development of the calvaria), and small eye socket (Table V-6). In the most severely affected fetuses from the high-dose groups, the anterior fontanelle, frontals, parietals, and/or nasals were fused, the zygomatics were short and/or flat, and the skull was abnormally shaped and small with incomplete ossification of the nasals and hypoplasia of the nasal portion of the eye sockets and communication of the sockets. Also frequently noted in these fetuses were hemivertebrae; fused, asymmetric, or unilaterally ossified centra; and short or absent tails. Less frequently noted were macrophthalmia, meningocele, cleft lip and/or palate, protruding tongue, and gastroschisis. Significant increases ($p < 0.01$) in the incidence of delayed ossification of the parietals was noted in fetuses from dams treated percutaneously with 3 mg/kg or higher of dinoseb.

The NOAEL for both maternal and developmental toxicity for this study was 1 mg/kg.

Table V-5. Summary of Embryo/Fetal Toxicity in Pregnant Rabbits Percutaneously Treated With Dinoseb During Days 7 to 19 of Gestation

Parameter	Dosage level (mg/kg)				
	0	1	3	9	9(18)
No. tested	17	17	17	21	8
No. pregnant (%)	16(94.1)	16(94.1)	16(94.1)	17(81.8)	8(100)
No. aborted	2	4	2	2	0
No. surviving and pregnant	10	10	11	3	1
No. live fetuses/litter	6.5 ± 2.8^a	7.1 ± 3.1	8.3 ± 2.3	3.3 ± 2.1	7.0 ± 0.0
No. dead fetuses	0	0	0	0	0
No. resorptions					
Early	0.8 ± 0.9^a	0.1 ± 0.3	0.5 ± 0.7	4.0 ± 3.0	0.8 ± 0.8
Late	0.1 ± 0.3^a	0.1 ± 0.3	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
Mean fetal body weight/litter (g)	42.2 ± 8.5^a	39.3 ± 7.4	37.2 ± 4.7	48.1 ± 5.0	- ^b

^aMean \pm SD.

^bFetal body weights inadvertently not recorded (one dam affected).

Table V-6. Incidences (%) of Litter and Fetal Malformations Found in Rabbits Percutaneously Treated With Dinoseb During Days 7 to 19 of Gestation

Tissue/ malformation	Dosage level (mg/kg)				
	1	1	3	9	9(18)
<u>Skull:</u>					
Microcephaly					
Litter	0	0	0	1(33.3)***	1(100)**
Fetal	0	0	0	1(10.0)**	6(85.7)**
Frontals fused					
Litter	0	0	2(18.2)	3(100)**	1(100)**
Fetal	0	0	3(3.3)	6(60)**	6(85.7)**
Eye sockets small					
Litter	0	0	1(9.1)	2(66.7)**	1(100)**
Fetal	0	0	1(1.1)	6(60.0)**	6(85.7)
Craniosynostosis					
Litter	0	0	2(18.2)	3(100)**	1(100)**
Fetal	0	0	3(3.3)	8(80.0)**	6(85.7)**
<u>Eyes:</u>					
Microphthalmia					
Litter	1(10.0)	0	0	2(66.7)**	1(100)**
Fetal	1(1.5)	0	0	5(50.0)**	7(100)**
Anophthalmia					
Litter	0	0	1(9.1)	3(100)**	1(100)**
Fetal	0	0	1(1.1)	3(30.0)**	1(14.3)**
Bulge, depressed, reduced, and/or ectopic					
Litter	1(10)	0	0	3(100)**	1(100)**
Fetal	1(1.5)	0	0	6(60.0)**	7(100)**
<u>Brain:</u>					
Hydrocephaly					
Litter	0	0	2(18.2)	3(100)**	7(100)**
Fetal	0	0	2(2.2)	7(70.0)**	6(85.7)**

*Number affected (incidence).

**Significantly different from controls ($p \leq 0.01$).

SOURCE: Hoberman (1987).

2. Reproductive Effects

Groups of 25 male and female rats in each of three generations received dinoseb (purity 98.4%) in the diet at dose levels of 0, 1, 3, and 10 mg/kg/day for 29 weeks (Irvine and Armitage, 1981). Both sexes at the high-dose level showed a lower rate of body weight gain in all three generations. This effect was inconsistent at lower dose levels and across generations. Dinoseb at the 10-mg/kg/day level elicited no effects on survival, fertility, or fecundity, and no microscopic or macroscopic changes; however, the numbers of pups born and pup weights at weaning were decreased at all dose levels and were attributed to maternal toxicity (lower maternal body weight). Findings at the lower dose levels, including minor skeletal defects in the F_0 generation, were considered incidental. A LOAEL of 1 mg/kg/day was identified.

An additional two generations were studied as a continuation of the above three-generation study (i.e., five consecutive generations were studied); the same doses were administered (Irvine and Armitage, 1981). Reduced gains in high-dose parental body weights persisted in the F_3 and F_4 generations. F_4 litter sizes at birth were reduced at 10 mg/kg, and F_4 preweaning pup weights were slightly reduced at 3 and 10 mg/kg. No progeny effects were noted for the F_5 generation. A LOAEL of 1 mg/kg/day was calculated.

Linder et al. (1982) studied the effects of DNB (purity of 97.3%) feeding on spermatogenesis in male rats. Technical grade dinoseb was fed for 11 weeks to adult male Sherman rats (99 to 115 days of age) at dosage levels of 75, 150, 225, or 300 ppm. On the basis of food consumption data, the authors calculated that these dosage levels averaged 3.8, 9.1, 15.6, or 22.2 mg/kg/day, respectively. Both a normal control group and a pair-weight control group (matched in body weight and pair-fed with the high-dose group) were included in the study. Thirty-six rats were fed at dietary levels of 0 and 22.2 mg/kg/day. All other groups consisted of 20 rats each. Four rats from each of the groups fed 0 or 22.2 mg/kg were sacrificed after 10, 20, 30, or 50 days of treatment. Half of the remainder in each group were sacrificed

during the 11th week (71 to 77 days). The remaining rats were utilized for reproductive and recovery studies. In rats fed 22.2 mg/kg/day, differential classification of spermatozoa from the cauda epididymis indicated that 90% of the sperm were atypical after 20 days of treatment. By 30 days, "bizarre" and amorphous forms were found, and epididymal sperm counts were decreased. Changes in the testes included abnormal spermatozoa and spermatids, multi-nucleated spermatogenic cells at 20 and 30 days, and severe damage to spermatogenic cells by 50 days. Dietary levels of 225 or 300 ppm produced marked oligospermia and extensive loss of spermatogenic cells in rats fed dinoseb for 11 weeks. Evidence of necrotic spermatogenic cells was seen in some tubules, and only Sertoli cells remained in many tubules. Reproductive failure occurred at 225 and 300 ppm, although mating behavior and libido appeared normal.

Little or no recovery was seen during a 16-week period after 300-ppm exposure was discontinued. The decrease in sperm count produced by doses of 150 or 225 ppm (9.1 or 15.6 mg/kg/day, respectively) appeared to be at least partly reversible with time. Sperm counts of rats at the various dietary levels after 71 to 77 days of exposure and after a 16-week recovery period are shown in Table V-7. No effects of any type were seen in the animals fed 75 ppm (3.8 mg/kg/day).

In contrast, Osterloh et al. (1983) evaluated testicular toxicity of dinoseb (purity of 98%) in male mice over a range of seven dose levels, and no effects were seen in any of the testicular parameters measured. Measurements of testicular effect included sperm morphology, sperm count, and testicular weight. Male hybrid (C57BL/6 x C3H)F₁ mice (7 to 10 weeks of age, four mice per dose level) were injected intraperitoneally with 2.0, 4.3, 9.3, 20, or 43 mg/kg/day or administered commercial grade dinoseb (98% pure) in corn oil, by gavage, at 20 mg/kg/day for 5 consecutive days. On day 35, the animals were killed, and the testes and cauda epididymides were removed for study. Total sperm counts were carried out on the epididymal sperm suspensions, and portions were stained with Eosin Y for morphological examination. All mice intraperitoneally administered 43 mg/kg/day died, and no effects on testicular

Table V-7. Epididymal Sperm Counts in Rats Fed Dinoseb for 71 to 77 Days

Weeks after dis- continuation of exposure	Dietary level (ppm)	Rats (n)	Epididymal fluid ^a		Sperm content of caudae and vasa deferentia	
			Epididymides (n)	Sperm count ^{b,c} (10/mg fluid)	Rats (n)	Sperm cells (10 ⁶)
0	0	9	16	1.45 ± 0.05	9	379 ± 40
	75	10	19	1.57 ± 0.07	10	458 ± 28
	150	10	18	1.21 ± 0.09 ^d	10	206 ± 1 ^{d,f}
	225	8	16	0.05 ± 0.02 ^d	9	20 ± 4 ^{d,f}
	300	3	5	0 ^d	5	9 ± 6
	0 ^e	9	16	1.54 ± 0.09	9	173 ± 29 ^d
16	0	10	18	1.53 ± 0.09	10	369 ± 36
	75	10	19	1.52 ± 0.13	10	402 ± 60
	150	10	19	1.55 ± 0.09	9	352 ± 47
	225	5	8	0.70 ± 0.24 ^d	10	78 ± 5 ^{d,f}
	300	1	1	0 ^d	4	6 ± 6 ^f
	0 ^e	10	19	1.50 ± 0.06	10	404 ± 36

^aSamples of less than 1 mg excluded; epididymal sample used as unit for calculations.

^bValues are group mean ± SEM.

^cZero values do not indicate complete azoospermia, only that no sperm cells were present in enumerated squares.

^dDiffers from control (p < 0.05).

^ePair-fed controls, given food equal to amount consumed by high-dose group.

^fAdjusted count (adjusted for the weight of the caudae and vasa deferentia) differs from control (p < 0.05).

SOURCE: Adapted from Linder et al. (1982).

parameters were observed at the other dose levels. The authors suggested several possibilities to account for the failure of DNBP to show effects in this study. Although some dosage levels were comparable to those reported in the study by Linder et al. (1982), the animals received only five daily doses, as compared to continuous daily ingestion over an 11-week period in the Linder report. In addition, since rats were used in the Linder study, species differences may also have been a factor in production of varying results.

D. MUTAGENICITY

Relatively few studies investigating the genotoxic potential of dinoseb have appeared in the published literature. This section includes the published experiments as well as a series of unpublished assays performed to meet U.S. EPA registration requirements. These are categorized into genemutation assays (Category 1), chromosome aberration assays (Category 2, none found for dinoseb), and studies that assess other mutagenic mechanisms (Category 3). The findings are discussed below.

1. Gene Mutation Assays (Category 1)

a. Reverse mutations in bacteria

Dinoseb (purity of 97.7%) elicited negative response when tested for mutagenicity in the Ames assay employing four strains (TA1535, TA1537, TA1538, and TA100) of Salmonella typhimurium and one strain (WP2 uvrA⁻) of Escherichia coli (Simmon et al., 1977). The assays were conducted in the absence or presence of a metabolic activation system derived from an Aroclor 1254-stimulated, rat liver homogenate. The Salmonella assays measured reversion to histidine prototrophy, and the E. coli assays measured reversion of WP2 to tryptophan independence.

Moriya et al. (1983) reported that dinoseb (purity not given) acetate was not mutagenic in bacterial reversion assay systems with five strains (TA100, TA98, TA1535, TA1537, and TA1538) of S. typhimurium and one strain (WP2 hcr) of E. coli. Dinoseb, along with 228 other pesticides, was tested with or

without S9 mix at doses up to 5,000 μ g/plate (unless the compound showed toxicity to bacteria at this dose).

Waters et al. (1982) tested dinoseb (purity not given) for mutagenic potential in the Ames assay using S. typhimurium and E. coli and reported that the herbicide was nonmutagenic. The assays were conducted both without and with a metabolic activation system derived from Aroclor 1254-induced rat livers. Five strains of S. typhimurium (TA1535, TA1537, TA1538, TA98, and TA100) and one strain of E. coli (WP2 uvrA) were used in these assays. Dinoseb was tested along with a large number of other pesticides. Each chemical was usually tested at a minimum of six concentrations; the highest nontoxic concentration tested was 10 mg/plate unless the chemical solubility dictated otherwise.

b. Sex-linked recessive lethals (SLRL) in Drosophila

Waters et al. (1982) reported that dinoseb (purity not given) did not induce sex-linked recessive lethals in Drosophila melanogaster. Doses tested were not reported. A compound was considered nonmutagenic if it did not elicit a 0.2% increase in mutation rate over the background and if the sample population tested was sufficient to permit detection at the 95% confidence level.

c. Mammalian cells in culture

Technical grade dinoseb, 4 to 48 μ g/mL without activation and 1.5 to 90 μ g/mL with activation, was tested in the L5178Y mouse lymphoma cell line for gene mutations (Den Boer, 1986). Without activation, small increases in mutation frequencies were induced at higher concentrations, just exceeding the minimum criteria for mutagenesis and thus indicating a weak mutagen. With activation, large increases in mutation frequency accompanied by high toxicity were noted. These increases could not be repeated but resulted in weakly positive results. The authors, therefore, concluded the material to be weakly positive.

2. Other Genotoxic Effects (Category 3)

a. Differential toxicity in bacteria

Dinoseb (purity of 97.7%) elicited positive response in DNA repair synthesis assay using repair-deficient and repair-proficient strains of E. coli (W3110 and p3478) and Bacillus subtilis (H17 and M45) (Simmon et al., 1977). Both solvent and positive controls were run concurrently. Newell (1981) reported that dinoseb (purity not given) was mutagenic in the DNA repair synthesis assay with E. coli (polA) but not mutagenic with B. subtilis.

Waters (1982) reported that dinoseb (purity not given) was classified as positive in three tests for primary DNA damage in prokaryotes. Differential toxicity assays were conducted with E. coli strains p3478 (DNA polymerase I-deficient polA⁻) and W3100; B. subtilis strains M45 (recombination-deficient recA⁻) and H17; and S. typhimurium strains SL4525 (rec⁺), SL4700 (rec⁻), TA1978, and TA1538. A positive response was indicated by a larger zone of inhibition on a repair-deficient strain than on the normal strain. Doses tested were not reported.

b. Mitotic recombination

Dinoseb (purity of 97.7%) was nonmutagenic in a mitotic recombination assay with Saccharomyces cerevisiae D3 (Simmons et al., 1977). The compound was tested at concentrations of 0.1, 0.2, and 0.3% (w/v or v/v) either without or with metabolic activation system from Aroclor 1254-induced rat livers. Both positive and negative controls were run concurrently. Newell (1981) also found dinoseb to be nonmutagenic in a mitotic recombination assay with S. cerevisiae D3.

Waters et al. (1982) reported that dinoseb (purity not given) did not induce mitotic recombination in S. cerevisiae D3. Five concentrations (not specified) of the test chemical were tested both with and without metabolic activation. A positive response was indicated by dose-related increases of

more than threefold in the absolute number of mitotic recombinants per mL and in the relative number of mitotic recombinations per 10^5 survivors.

c. Unscheduled DNA synthesis (UDS)

Dinoseb (purity of 97.7%) elicited negative response in the UDS assay either without (dose range 10^{-7} to 10^{-4} M) or with (dose range 10^{-5} to 10^{-3} M) the metabolic activating system (Simmons et al., 1977). Five replicate cultures of diploid WI-38 human fibroblast cells were used for the UDS assay. Both solvent and positive controls were run concurrently. Mitchell (1981) also reported that dinoseb (purity not given) was not mutagenic in the UDS assay with diploid human fibroblasts (WI-38 cells); doses used were not reported.

E. CARCINOGENICITY

In a 2-year feeding study conducted by Hazleton (1977), groups of 60 albino rats per sex were administered dinoseb at 0, 1, 3, or 10 mg/kg/day in the diet. No increased incidences of tumors were found in high-dose rats compared to controls. However, tissues from only 10 animals per sex from the control and high-dose group in addition to the liver, kidneys, and lesions from animals in the low- and mid-dose groups were examined at the interim sacrifice (week 52) and terminal sacrifice (week 104).

Brown (1981) conducted a carcinogenicity study with groups of 70 male and 70 female CD mice administered 0, 1, 3, or 10 mg/kg/day of technical grade dinoseb (purity not given) in the diet for 100 weeks.

Female mice showed an increased (but not dose-related) incidence of liver adenomas and combined adenomas and carcinomas (Table V-8). Dinoseb induced statistically significant ($p < 0.05$) increases in liver adenomas in female mice at the 3- and 10-mg/kg/day doses. The incidence for adenomas was 0/57, 3/59, 7/60, and 5/58 for the 0-, 1-, 3-, and 10-mg/kg/day doses, respectively. Only one hepatocellular carcinoma was observed in female mice; this occurred in the low-dose group. No adenomas or carcinomas were noted in the

In rats administered dinoseb at dietary levels of 13.5 mg/kg/day for 21 days, blood urea nitrogen increased to 55 mg% versus 19.4 mg% in the controls. This was accompanied with slight degenerative changes in the renal tubules and cloudy swelling in the liver. Dinoseb administered intraperitoneally at doses of 12 to 16 mg/kg/day for 5 days intensified inhibitory and excitatory activities in the brains of rats, and daily doses of 2 to 8 mg/kg/day for 5 days were without effect.

Using the duckling as an experimental model, it has been demonstrated that dinoseb, in common with a number of other dinitrophenols, has the ability to produce cataracts following dietary exposure.

Dinoseb, administered at dietary levels of 300 ppm and above to rats for 60 days, resulted in a high incidence of death. Depressed growth was noted at the lower dose levels (50 to 200 ppm) as were decreased organ weights and lactic dehydrogenase and cholinesterase activities. Increases were seen in organ-to-body weight ratios, alkaline phosphatase, alanine aminotransferase, potassium, and urea nitrogen. Discrimination learning was not affected. Diffuse tubular atrophy of the testes was noted, especially at the 200-ppm level.

In a 6-month feeding study, the body weights of rats receiving 5.4 mg/kg/day were slightly lower than those of controls at the end of the treatment period. No changes were noted in hematology gross examination, mean organ weights, and histopathology, except for a slight but statistically significant increase in mean liver weight. Dietary levels of 13.5 mg/kg/day resulted in increased mortality.

Beagle dogs administered 0.01 or 0.005% dinoseb in the diet for 91 days were without adverse effects, and those administered 0.02 and 0.03% levels in the diet showed decreased body weight gain, increased average liver weights, mural endocarditis, and microscopic heart changes in females only. The NOAEL was 0.01% (100 ppm), equivalent to 4 mg/kg/day.

In a chronic toxicity study conducted in rats fed 1, 3, and 10 mg/kg/day in the diet for 2 years, a compound-related decrease in mean thyroid weights was reported. No other compound-related effects were observed; however, histopathologic evaluation of tissues was conducted in only a limited number of animals. The results suggest a LOAEL of 1 mg/kg/day.

Mice orally administered dinoseb in the diet for 100 weeks, at 1, 3, and 10 mg/kg/day, showed cystic endometrial hyperplasia and atrophy, hypospermatogenesis, and testicular degeneration. Lenticular opacities were noted at the 3- and 10-mg/kg/day dose levels; the lowest level was not examined for this effect. A systemic NOAEL is less than 1 mg/kg/day.

Dinoseb has been found to cause skeletal anomalies in fetuses of several species following oral, intraperitoneal, subcutaneous, and dermal administration to pregnant animals. Oral administration of dinoseb to mice on days 10 to 12 of gestation produced skeletal anomalies at 20 and 32 mg/kg/day; maternal mortality was also present at these dose levels. However, in mice orally administered 15 and 100 mg/kg during days 8 through 12 of gestation, no effects were seen on postnatal parameters at day 22, 30, or 57. Other studies suggest that the rat may be more susceptible than the mouse to the effects of dinoseb. Pregnant Sprague-Dawley rats fed 8.6 mg/kg/day or more of dinoseb in the diet on days 6 to 15 of gestation exhibited poor weight gain, sometimes with ataxia, and lethargy. Fetal survival was decreased at and above doses of 6.9 mg/kg/day; decreases reached significance at or above 8.6 mg/kg/day.

Oral administration of dinoseb to mice on day 8 of gestation at doses of 26 and 33 mg/kg produced supernumerary ribs. The same finding was seen in rats administered 10 mg/kg dinoseb on days 6 through 15 of gestation. Skeletal anomalies were also observed in rabbits orally administered 10 mg/kg dinoseb on days 6 through 18 of gestation, as were external and visceral malformations.

Dinoseb appears to elicit an even greater incidence of developmental anomalies after dermal exposure in pregnant rabbits. Increased incidences of gross external, soft tissue, and skeletal malformations were noted in fetuses

of rabbits percutaneously treated with dinoseb at 3 mg/kg/day or higher. These malformations included hydrocephaly, microphthalmia, anophthalmia, cranio-synostosis, and small eye sockets. The NOAEL for maternal toxicity was also 1 mg/kg/day, based on mortality, slight decreases in body weight during the dosing period, and increased incidences of gross lesions upon necropsy of rabbits receiving dosages of 3 mg/kg/day or higher.

Treatment of pregnant mice with 17.7 mg/kg dinoseb administered intraperitoneally on days 10 to 12 of gestation resulted in a variety of fetal defects, including fused or missing ribs, fused or missing sternbrae, fused or unossified or absent vertebrae, and absent or unossified long bones. Although doses of 10 to 15.8 mg/kg had no maternal or developmental effects when administered on gestation days 10 to 12, 12.5 mg/kg/day on days 14 to 16 significantly increased resorption rates and reduced fetal weights.

In a study of postnatal morphology and functional capacity of kidneys in neonates of Sprague-Dawley rats treated intraperitoneally with dinoseb, it was demonstrated that approximately 40% of the fetuses of mothers intraperitoneally administered dinoseb at 8.0 to 9.0 mg/kg/day had dilated renal pelvis and/or ureters. Histological examination revealed relatively complete recovery when offspring were examined at 6 weeks of age. In contrast, livers of fetuses from this same group showed highly vacuolated cells on initial examination; these toxic effects were still present in the livers of the offspring 6 weeks later, along with necrotic cells and pyknotic or karyorrhectic nuclei in other cells. Thus, the liver showed little evidence of recovery from the initial damage.

It has been found that pretreatment of pregnant mice with SKF-525A (a mixed-function oxidase inhibitor) potentiates resorptions and reductions in fetal body weights induced by dinoseb when injected intraperitoneally. In contrast, pretreatment with phenobarbital, which stimulates the hepatic mixed function oxidase system, inhibits these effects.

In rat studies in which dinoseb was administered in the diet for five consecutive generations at 1, 3, and 10 mg/kg/day, no effects on survival,

fecundity, or fertility were seen. At 10 mg/kg/day, the litter sizes at birth and the pup weights at weaning were reduced; this was attributed to maternal toxicity.

Dietary levels of dinoseb at 15.6 or 22.2 mg/kg/day for 11 weeks produced marked oligospermia and extensive loss of spermatogenic cells in the testes of rats. Little recovery occurred during the 16 weeks following cessation of exposure. At a dose level of 9.1 mg/kg/day, decreased epididymal sperm counts, atypical epididymal spermatozoa, and minimal testicular changes were present. These effects appeared to be reversible with time. No effects were seen in the rats fed 3.8 mg/kg/day in this 11-week study.

No testicular effects were noted, however, in mice receiving oral or intraperitoneal doses of up to 20 mg/kg/day for 5 consecutive days. Intraperitoneal doses of 43 mg/kg/day were lethal.

One report suggested that orally administered dinoseb (about 20 mg/kg) may have long-term inhibitory effects on both the cellular and humoral immune responses in the hamster.

A number of assays were conducted to determine the mutagenic potential of dinoseb. Negative responses were elicited in the Ames assay with S. typhimurium and E. coli, sex-linked recessive lethal assay in D. melanogaster, mitotic recombination assay in S. cerevisiae, and unscheduled DNA synthesis assay in human fibroblasts. However, positive responses were elicited in DNA repair synthesis assays using repair-deficient and repair-proficient strains of E. coli, B. subtilis, and S. typhimurium. Dinoseb also induced small increases in mutation frequencies in a mouse lymphoma cell line.

No increases in tumor incidences were observed in rats fed dinoseb at levels of 0, 1, 3, or 10 mg/kg/day in the diet for 104 weeks. However, only a limited number of animals were examined histologically. Mice administered dinoseb orally in the diet for 100 weeks at 1, 3, and 10 mg/kg/day showed equivocal oncogenic effects, although statistically significant increases in

the incidence of liver adenomas and combined adenomas and carcinomas were observed in female mice only.

VI. HEALTH EFFECTS IN HUMANS

A. CLINICAL CASE STUDIES

Smith (1981) reported a case history of an individual apparently poisoned by a dinoseb-containing herbicide. A self-employed farmer unfamiliar with use of the herbicide sprayed an area of new grass seed with the product. During spray operations, he unplugged a spray jet with his bare hands. He wore a gauze face mask, which he noted was heavily stained yellow at the end of the spraying operation. Later that day, the farmer developed a headache, malaise, lassitude, and sweating. The next day, he sought medical advice at the nearest hospital casualty department. After a conference with a member of the manufacturer's medical department, and in view of the minimal exposure and the clinical profile, the joint tentative diagnosis was that the patient was suffering from influenza, and he was referred to the care of his general practitioner.

Over the subsequent 5 days, the patient had anorexia, bouts of excessive sweating and intermittent shivering, pains in the chest and abdomen, excessive thirst, restlessness, insomnia, loose stool, and weight loss (10 kg during the week). He developed further symptoms of respiratory involvement, including shortness of breath and hemoptysis, and displayed personality changes that alarmed the family. Six days after the incident, the farmer was seen by his general practitioner, who referred him to the hospital where he was admitted. On admission, the patient was flushed, with a temperature of 39.8°C. He exhibited intermittent dyspnea, spasmodic coughing, dullness at the base of one lung, and crepitations. He was immediately treated with oxytetracycline. The patient complained of photophobia and some neck stiffness and was found to have a positive Kernig sign. His erythrocyte sedimentation rate was 96 mm/hour. The urine was discolored yellow. A blood sample was collected, analyzed for dinitro compounds, and found to be negative. Liver function was impaired, and chest X-ray revealed patchy shadowing at the bases. Lung function tests indicated considerable impairment.

At the end of 1 week, the farmer's clinical picture had improved sufficiently to permit his discharge from the hospital; however, his liver function test was more abnormal than it had been at the time of admission. Two weeks later, as an outpatient, he was still complaining of lethargy, night sweats, and forgetfulness. His condition slowly improved, and after 10 to 12 weeks he was symptom free. However, 6 months after the incident, his blood urea was reported to be 7.9 mmol/L (the normal range is 3.5 to 6.5 mmol/L). The author of this report suggested that both inhalation and skin exposure may have played a role in the toxicity. It is interesting to note that in animal studies by McCormack et al. (1980) (see Section V.C.1.b), offspring of dinoseb-exposed rats showed kidney damage from which they recovered by 42 days postpartum, but liver damage had not improved and may have been worse. This finding is consistent with the case study reported above.

Heyndrickx et al. (1964) reported a fatal human exposure to two herbicides, Nitrador 40 (40% dinitro-ortho-cresol) and Dinorsol PL (14% dinoseb). Five days after spraying with these two herbicides, a farm worker suddenly became ill. He vomited frequently and felt tired. The following day he complained of violent spasms, intense thirst, and tachycardia. He was hospitalized, and he died the following day. At autopsy, no specific cause of death could be ascertained. Dinitro-ortho-cresol was identified from the skin of the hand but could not be identified in other tissues. An unidentified metabolite was found in urine. Both herbicides could be identified in extracts of the overalls, cap, and mask. The authors point out that delayed effects in humans have been previously reported for dinitro-ortho-cresol poisoning. The contribution of dinoseb to the toxic response in this incident cannot be determined, although the analysis of clothing indicated that about twice as much dinoseb as dinitro-ortho-cresol was present at the time of analysis.

B. EPIDEMIOLOGICAL STUDIES

No epidemiological studies of dinoseb were found.

more than threefold in the absolute number of mitotic recombinants per mL and in the relative number of mitotic recombinations per 10^5 survivors.

c. Unscheduled DNA synthesis (UDS)

Dinoseb (purity of 97.7%) elicited negative response in the UDS assay either without (dose range 10^{-7} to 10^{-4} M) or with (dose range 10^{-5} to 10^{-3} M) the metabolic activating system (Simmons et al., 1977). Five replicate cultures of diploid WI-38 human fibroblast cells were used for the UDS assay. Both solvent and positive controls were run concurrently. Mitchell (1981) also reported that dinoseb (purity not given) was not mutagenic in the UDS assay with diploid human fibroblasts (WI-38 cells); doses used were not reported.

E. CARCINOGENICITY

In a 2-year feeding study conducted by Hazleton (1977), groups of 60 albino rats per sex were administered dinoseb at 0, 1, 3, or 10 mg/kg/day in the diet. No increased incidences of tumors were found in high-dose rats compared to controls. However, tissues from only 10 animals per sex from the control and high-dose group in addition to the liver, kidneys, and lesions from animals in the low- and mid-dose groups were examined at the interim sacrifice (week 52) and terminal sacrifice (week 104).

Brown (1981) conducted a carcinogenicity study with groups of 70 male and 70 female CD mice administered 0, 1, 3, or 10 mg/kg/day of technical grade dinoseb (purity not given) in the diet for 100 weeks.

Female mice showed an increased (but not dose-related) incidence of liver adenomas and combined adenomas and carcinomas (Table V-8). Dinoseb induced statistically significant ($p < 0.05$) increases in liver adenomas in female mice at the 3- and 10-mg/kg/day doses. The incidence for adenomas was 0/57, 3/59, 7/60, and 5/58 for the 0-, 1-, 3-, and 10-mg/kg/day doses, respectively. Only one hepatocellular carcinoma was observed in female mice; this occurred in the low-dose group. No adenomas or carcinomas were noted in the

Table V-8. Incidence of Hepatocellular Adenoma and Carcinoma in Mice Receiving Dinoseb in the Diet for 100 Weeks

Neoplasm	Dosage level (mg/kg)							
	0 (control)		1		3		10	
	M	F	M	F	M	F	M	F
No. of livers examined	70	70	70	70	70	70	70	70
Hepatocellular adenoma	11	0	16	3	17	7	16	5
Hepatocellular carcinoma	5	0	4	1	9	0	5	0

SOURCE: Adapted from Brown (1981).

female controls. No statistical difference from controls was observed in the incidence of hepatocellular adenoma and carcinoma in males at any level. No biological significance was attributed to the increased occurrence of these adenomas in females, since there was no dose relationship, only a low number of animals was affected, and there was a lack of other hepatocellular changes commonly associated with carcinogens. In addition, the lack of any adenomas in the female controls is not consistent with the normal incidence in controls of this strain. It was concluded that there were no treatment-related neoplastic changes.

Further details on both studies were described in Section V.B.2, Chronic Effects.

In a separate screening study, mice failed to demonstrate any significant increase in tumors (Innes et al., 1969). Two strains of mice (hybrids of female C57BL/6 and male C3H/Anf or AKR mice, 18/sex/group) were exposed to dinoseb for 18 months. The animals were first exposed via gavage at 2.15 mg/kg/day for 3 weeks beginning at 1 week of age; then they were fed a diet containing 7 ppm dinoseb (1.05 mg/kg/day) throughout the observation period of approximately 18 months. Equal numbers of mice served as controls. After 18 months on diet, dinoseb did not cause any significant increase in tumors in mice.

F. SUMMARY

Acute oral LD_{50} values for the rat, mouse, rabbit, and guinea pig range from 14 to 114 mg/kg. The intraperitoneal LD_{50} value has been reported as 20.2 mg/kg for female mice and 10 mg/kg for male mice. Prostration, rapid respiration, and convulsions immediately preceding death were observed in guinea pigs receiving acute doses. Elevated environmental temperatures lowered the LD_{50} for female mice intraperitoneally injected with dinoseb. Dinoseb is apparently well absorbed through the intact skin, with dermal LD_{50} values in the rat ranging from 67 to 134 mg/kg; absorption depended to a large extent on the method of application and the covering employed.

In rats administered dinoseb at dietary levels of 13.5 mg/kg/day for 21 days, blood urea nitrogen increased to 55 mg% versus 19.4 mg% in the controls. This was accompanied with slight degenerative changes in the renal tubules and cloudy swelling in the liver. Dinoseb administered intraperitoneally at doses of 12 to 16 mg/kg/day for 5 days intensified inhibitory and excitatory activities in the brains of rats, and daily doses of 2 to 8 mg/kg/day for 5 days were without effect.

Using the duckling as an experimental model, it has been demonstrated that dinoseb, in common with a number of other dinitrophenols, has the ability to produce cataracts following dietary exposure.

Dinoseb, administered at dietary levels of 300 ppm and above to rats for 60 days, resulted in a high incidence of death. Depressed growth was noted at the lower dose levels (50 to 200 ppm) as were decreased organ weights and lactic dehydrogenase and cholinesterase activities. Increases were seen in organ-to-body weight ratios, alkaline phosphatase, alanine aminotransferase, potassium, and urea nitrogen. Discrimination learning was not affected. Diffuse tubular atrophy of the testes was noted, especially at the 200-ppm level.

In a 6-month feeding study, the body weights of rats receiving 5.4 mg/kg/day were slightly lower than those of controls at the end of the treatment period. No changes were noted in hematology gross examination, mean organ weights, and histopathology, except for a slight but statistically significant increase in mean liver weight. Dietary levels of 13.5 mg/kg/day resulted in increased mortality.

Beagle dogs administered 0.01 or 0.005% dinoseb in the diet for 91 days were without adverse effects, and those administered 0.02 and 0.03% levels in the diet showed decreased body weight gain, increased average liver weights, mural endocarditis, and microscopic heart changes in females only. The NOAEL was 0.01% (100 ppm), equivalent to 4 mg/kg/day.

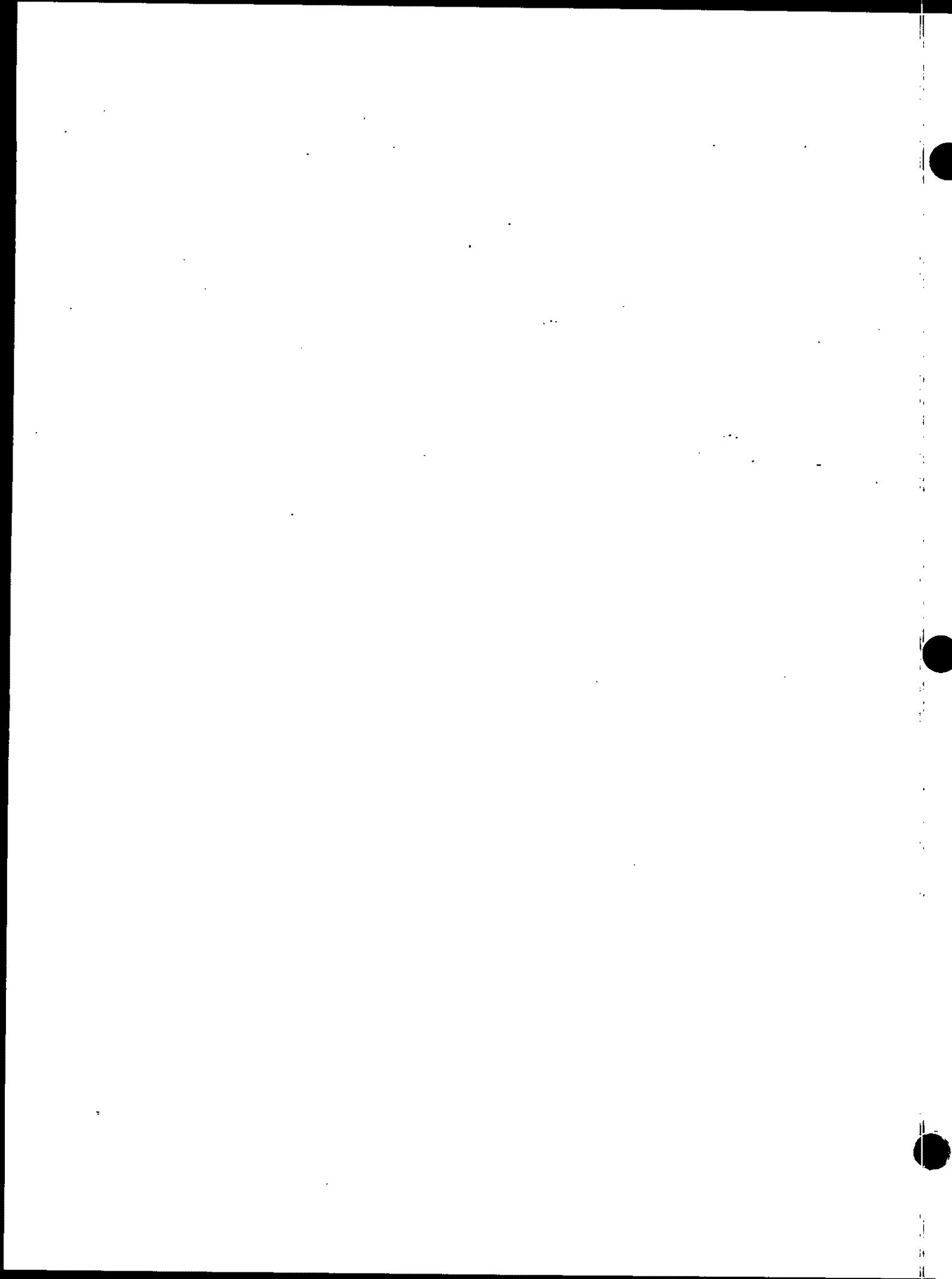
C. HIGH-RISK POPULATIONS

No specific subpopulations were identified that appear to be at greater risk from dinoseb exposure than the general population. Certain work populations, including those involved in manufacture and, perhaps to a greater extent, those who mix and apply this herbicide in the field, have the potential for greater exposure.

D. SUMMARY

One clinical case history of dinoseb poisoning, from which the patient apparently recovered, has been reported in the literature. Elevated body temperature, liver damage, and subsequent lung involvement were the major effects. The liver damage appeared to be particularly long lasting. This has also been reported in experimental animals (see Chapter V of this report).

No relevant epidemiological studies were found, and no high-risk populations were identified.



VII. MECHANISMS OF TOXICITY

A. UNCOUPLING OF OXIDATIVE PHOSPHORYLATION

Ilivicky and Casida (1969) reported an extensive investigation of the uncoupling of oxidative phosphorylation in mitochondria by five dinitrophenol compounds, including dinoseb. Mitochondria were prepared from the brains and livers of male albino mice (18 to 20 g, strain unspecified). The potency of the uncoupling action was judged by the minimum concentration of chemical necessary for complete uncoupling. Brain mitochondria were up to 50-fold more sensitive to the uncoupling action of the 2,4-dinitrophenols than were liver mitochondria. The minimum uncoupling concentration for dinoseb with mouse liver mitochondria was 1.0 μ M compared with 50 μ M for 2,4-dinitrophenol. With brain mitochondria, the differences were 0.5 μ M for dinoseb versus 1.0 μ M for 2,4-dinitrophenol. In both systems, dinoseb was the most potent uncoupler of the five dinitrophenols studied.

Ilivicky and Casida (1969) also studied the effect of dinoseb on mitochondrial respiration, when the chemical was administered in vivo. Groups of six mice were injected intraperitoneally with dinoseb at doses of 15, 24, or 36 mg/kg. Animals were observed for 20 minutes to note the resulting severity of symptoms; mitochondria were prepared from the brains and livers of sacrificed animals. A dose of 15 mg/kg produced no external symptoms, and the isolated liver and brain mitochondria were not uncoupled. At a dose of 24 mg/kg, mild external symptoms occurred in animals, and partial uncoupling was observed in brain and liver mitochondria. A dose of 36 mg/kg produced severe manifestations of toxicity, and complete uncoupling of oxidative phosphorylation was observed in both the liver and brain. Symptoms of toxicity were dyspnea, weakness, occasional salivation, and death when poisoning was severe. With one exception, severity of the toxic response paralleled the degree of uncoupling of brain mitochondria for dinoseb and 21 other known uncouplers of oxidative phosphorylation. According to the authors, the data provide strong evidence that the uncoupling of oxidative phosphorylation may be the mechanism of action of dinoseb.

B. METHEMOGLOBIN FORMATION

Dinoseb produces considerable methemoglobin in ruminants, although methemoglobin formation is minimal in other species exposed to the herbicide (Froslic and Karlog, 1970; Froslic, 1974, 1976). These authors suggested that methemoglobin formation is related to the formation of the diamino metabolite of dinoseb in the rumen of these animals. Froslic (1976) found that elevated methemoglobin levels produced in seven sheep after intraruminal administration of nitrite lasted only about 12 hours, and dinoseb-induced methemoglobinemia persisted for at least 2 to 3 days. The dinoseb effect correlated with a progressive and almost complete inhibition of NADH-methemoglobin reductase in the red blood cells. This inhibition may underlie the methemoglobinemia found in the ruminant after ingestion of dinoseb.

C. INTERACTIONS

In a study discussed in detail in this report (Section V.C.1.b), Preache and Gibson (1975b) found that SKF-525A potentiated the resorptions and reductions in fetal body weight induced by dinoseb. In contrast, pretreatment of pregnant rats with phenobarbital inhibited these effects.

D. SUMMARY

Dinoseb, like other dinitrophenols, is an uncoupler of oxidative phosphorylation, particularly in brain mitochondria. Dinoseb inhibition of brain oxidative phosphorylation correlates with signs and severity of toxicity. In animals showing severe external signs of poisoning, brain mitochondrial oxidative phosphorylation was completely inhibited.

Synergistic effects have been shown with SKF-525A, and antagonistic effects have been shown with phenobarbital following intraperitoneal administration in pregnant Swiss-Webster mice. The former potentiates and the latter inhibits resorptions and reductions in fetal body weights induced by dinoseb.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of noncarcinogenic and carcinogenic effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, whereas carcinogens are assumed to act without a threshold. The quantification of noncarcinogenic effects (One-, Ten-, Longer-term Health Advisories) that were calculated using suitable oral toxicity studies are presented in this chapter.

A. PROCEDURES FOR QUANTIFICATION OF TOXICOLOGICAL EFFECTS

1. Noncarcinogenic Effects

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI), is calculated. The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious health effects, even if exposure occurs over a lifetime. The RfD is derived from a No-Observed-Adverse-Effect Level (NOAEL) or Lowest-Observed-Adverse-Effect Level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor (UF). The RfD is calculated as follows:

$$\text{RfD} = \frac{(\text{NOAEL or LOAEL})}{\text{Uncertainty factor}} = \text{--- mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based on professional judgment while considering the entire data base of toxicological effects for the chemical. To ensure that uncertainty factors are selected and applied in a consistent manner, the Office of Drinking Water employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980), as follows:

- An uncertainty factor of 10 is generally used when good chronic or subchronic human exposure data identifying a NOAEL are available and are supported by good chronic or subchronic toxicity data for other species.
- An uncertainty factor of 100 is generally used when good chronic toxicity data identifying a NOAEL are available for one or more animal species (and human data are not available), or when good chronic or subchronic toxicity data identifying a LOAEL in humans are available.
- An uncertainty factor of 1,000 is generally used when limited or incomplete chronic or subchronic toxicity data are available, or when good chronic or subchronic toxicity data identifying a LOAEL, but not a NOAEL, for one or more animal species are available.

The uncertainty factor used for a specific risk assessment is based principally on scientific judgment, rather than scientific fact, and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less-than-lifetime study for deriving an RfD, the significance of the adverse health effect, and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium-specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not expected to occur. The DWEL assumes 100% exposure from drinking water. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{\text{RfD} \times (\text{body weight in kg})}{\text{Drinking water volume in L/day}} = \text{mg/L}$$

where:

Body weight = assumed to be 70 kg for an adult.

Drinking water volume = assumed to be 2 L per day for an adult.

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (One-day, Ten-day, and Longer-term HAs) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using a similar equation to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (\text{bw})}{(\text{L/day}) \times (\text{UF})} = \text{--- mg/L (--- } \mu\text{g/L)}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. One-day HA for a 10-kg child ingesting 1 L water per day.
2. Ten-day HA for a 10-kg child ingesting 1 L water per day.
3. Longer-term HA for a 10-kg child ingesting 1 L water per day.
4. Longer-term HA for a 70-kg adult ingesting 2 L water per day.

The One-day HA, calculated for a 10-kg child, assumes a single acute exposure to the chemical and is generally derived from a study of less than 7 days' duration. The Ten-day HA assumes a limited human exposure period of 1 to 2 weeks and is generally derived from a study of less than 30 days' duration. The Longer-term HA is calculated for both a 10-kg child and a 70-kg adult and assumes a human exposure period of approximately 7 years (or 10% of an individual's lifetime). The Longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of an animal's lifetime).

2. Carcinogenic Effects

The EPA categorizes the carcinogenic potential of a chemical, based on the overall weight of evidence, according to the following scheme:

- Group A: Known Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.
- Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.
- Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.
- Group D: Not Classified as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.
- Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable, or possible human carcinogen, mathematical models are used to calculate the estimate of excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies in animals. To predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than-lifetime studies, and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the

animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 liters of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure via ingestion of water. The cancer unit risk is usually derived from a linearized multistage model, with a 95% upper confidence limit providing a low-dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit, and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any others. Because each model is based on differing assumptions, the estimates that were derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water; the impact of the experimental animal's age, sex, and species; the nature of the target organ system(s) examined; and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure, not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

B. QUANTIFICATION OF NONCARCINOGENIC EFFECTS FOR DINOSEB

The following Health Advisories (One-day, Ten-day, and Longer-term), the Reference Dose, and the Drinking Water Equivalent Level were calculated based on ingestion data from suitable short- and long-term oral toxicity studies.

1. One-day Health Advisory

No suitable studies were found for calculating the One-day Health Advisory. U.S. EPA (1987) recommended that the Ten-day HA value of 0.3 mg/L for a 10-kg child (calculated below) be used as a conservative estimate of the One-day HA value.

2. Ten-day Health Advisory

In a developmental toxicity study with rabbits (Becker, 1986b), the oral administration of dinoseb (purity 98.1%) produced neural tube defects at doses greater than 3 mg/kg/day (NOAEL). The results of a recently completed developmental toxicity study (Hoberman, 1987), in which pregnant rabbits received percutaneous dosages of dinoseb, identified a developmental NOAEL of 1 mg/kg/day, based on increased incidences of gross external, soft tissue, and skeletal malformations in fetuses from dams receiving dosages of 3 mg/kg/day or higher. Although the Hoberman study (1987) identified a lower NOAEL, the oral study represents the more appropriate route of exposure. Data from an absorption study in monkeys (Bucks, 1987) suggest that a percutaneous dose of 0.2 mg/cm² results in the maximum absorption rate and higher doses do not result in greater absorption. Furthermore, a dermal absorption model developed by the Office of Drinking Water indicates that human exposure to dinoseb in the drinking water via the dermal route is negligible. Therefore, the developmental toxicity study of Becker (1986b) was selected as the basis for determination of the Ten-day HA. While it is reasonable to base the Ten-day HA for an adult on a positive developmental toxicity study, there is some question as to whether it is appropriate to base the Ten-day HA for a 10-kg child on such a study. However, since this study is of appropriate duration

and since the fetus may be more sensitive than a 10-kg child, it was judged that, while this number may be overly conservative, it is a reasonable basis for the Ten-day HA for a 10-kg child.

Using a NOAEL of 3 mg/kg/day, the Ten-day HA for a 10-kg child is calculated as follows:

$$\text{Ten-day HA} = \frac{(3 \text{ mg/kg/day})(10 \text{ kg})}{(100)(1 \text{ L/day})} = 0.3 \text{ mg/L (300 } \mu\text{g/L)}$$

where:

3 mg/kg/day = NOAEL, based on the absence of teratogenic effects in rabbits.

10 kg = assumed body weight of a child.

100 = uncertainty factor, chosen in accordance with NAS/CDV guidelines for use with a NOAEL from an animal study.

1 L/day = assumed daily water consumption of a child.

3. Longer-term Health Advisory

The Hall et al. (1978) 153-day dietary dinoseb (purity 80%) study in rats was originally selected to serve as the basis for determination of the Longer-term HA (decreased growth was observed at all exposure levels with a LOAEL of 2.5 mg/kg/day). Subsequently, however, a 3-generation reproduction study in rats (Irvine and Armitage, 1981) identified a LOAEL of 1 mg/kg/day (purity 98.4%), based on decreases in pup body weights at all dose levels. Since a reproduction study is of appropriate duration, the Irvine and Armitage (1981) study has been selected to serve as the basis for determination of the Longer-term HA.

Using a LOAEL of 1 mg/kg/day, the Longer-term HA for a 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(1 \text{ mg/kg/day})(10 \text{ kg})}{(1,000)(1 \text{ L/day})} = 0.010 \text{ mg/L (10 } \mu\text{g/L)}$$

where:

1 mg/kg/day = LOAEL, based on decreased pup body weight.

10 kg = assumed body weight of a child.

1,000 = uncertainty factor, chosen in accordance with NAS/ODW guidelines for use with a LOAEL from an animal study.

1 L/day = assumed daily water consumption of a child.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\text{Longer-term HA} = \frac{(1 \text{ mg/kg/day})(70 \text{ kg})}{(1,000)(2 \text{ L/day})} = 0.035 \text{ mg/L (40 } \mu\text{g/L)}$$

where:

1.0 mg/kg/day = LOAEL, based on decreased pup body weight.

70 kg = assumed body weight of an adult.

1,000 = uncertainty factor, chosen in accordance with NAS/ODW guidelines for use with a LOAEL from an animal study.

2 L/day = assumed daily water consumption of an adult.

4. Reference Dose and Drinking Water Equivalent Level

Originally, the 2-year dietary study in rats by Hazleton (1977) was selected to serve as the basis for determination of the Lifetime HA. In this study, a LOAEL of 1 mg/kg/day was identified based on a compound-related decrease in mean thyroid weights in all dosed males. However, tissues from only a limited number of animals were examined histopathologically. A more

complete histopathological examination of tissues from mice fed diets containing dinoseb for 100 weeks (Brown, 1981) also identified a LOAEL of 1 mg/kg/day. Cystic endometrial hyperplasia and atrophy, hypospermatogenesis, and degeneration of the testes were noted in females and males, respectively, receiving 1, 3, or 10 mg/kg/day. This LOAEL of 1 mg/kg/day was also supported by a three-generation reproductive study (Irvine and Armitage, 1981) which demonstrated decreased fetal weights and a decrease in pup body weights at all dose levels. Using a LOAEL of 1 mg/kg/day, the RfD for a 70-kg adult is calculated as follows:

Step 1: Determination of the Reference Dose (RfD)

$$\text{RfD} = \frac{(1 \text{ mg/kg/day})}{(1,000)} = 0.001 \text{ mg/kg/day}$$

where:

1 mg/kg/day = LOAEL, based on decreased thyroid weight in male rats and/or hypospermatogenesis, and degeneration of the testes and cystic endometrial hyperplasia and atrophy in male and female mice in chronic dietary studies and decreased fetal weights and a decrease in pup body weights in a three-generation reproductive study.

1,000 = uncertainty factor, chosen in accordance with NAS/CDW guide lines for use with a LOAEL from an animal study.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.001 \text{ mg/kg/day}) (70 \text{ kg})}{(2 \text{ L/day})} = 0.035 \text{ mg/L (40 } \mu\text{g/L)}$$

where:

0.001 mg/kg/day = RfD.

70 kg = assured body weight of an adult.

2 L/day = assured daily water consumption of an adult.

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